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THE ACETYLCHOLINE CONTENT AND CHOLINESTERASE ACTIVITIES IN THE RABBIT HEART IN EXPERIMENTAL HEART FAILURE AND THE EFFECT OF g-STROPHANTHIN TREATMENT UPON THEM

BY

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To my parents

FOREWORD

This investigation was made at the Department of Pharmacology of the University of Helsinki during the years 1956—1958. The subject was suggested by the Head of the Institute, Professor Armas Vartiainen, M.D., to whom I wish to express my thanks for his tireless interest. He has unhesitatingly placed all the facilities of the institute at my disposal.

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Pertti Torsti

LIST OF ABBREVIATIONS

ACh = Acetylcholine

BuCh = Butyrylcholine

ChE = Cholinesterase

MCh = Acetyl-β-methylcholine

CR = untreated control rabbit

HR = untreated rabbit with heart failure

SCR = control rabbit treated with g-strophanthin

SHR = rabbit with heart failure treated with g-strophanthin

IVS = interventricular septum

LA = left auricle

LV = left ventricle

RA = right auricle

RV = right ventricle

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I. INTRODUCTION

The mammal heart is of the so-called myogenic type, the rhythmic function of which depends on the properties of the heart muscle. The impulses triggering the contraction usually have their origin in pacemaker nodal tissue, which is a specialized heart muscle tissue. The neural factors, the opposite autonomic nervous systems, control the myogenic functioning of the heart rather than being a prerequisite for its automatism. The phenomena connected with the functioning of the heart can be divided into two categories: on the one hand, the rhythmicity of fts function, its excitability, the conductivity of the impulse and the refractoriness, and on the other hand the very process of contraction, *i.e.* the combination of actine and myosine into actomysine and the contraction of this through the energy stored in energy-rich phosphates. The characteristics of the muscle cell membrane are the basis for the originating of the pacemaker impulse, the conduction of the excitation and the releasing of the contraction. Heart failure is a syndrome which on the one hand can be explained by the fact that the tissues receive an insufficient amount of blood, in other words the minute volume of the heart is inadequate, and on the other hand by venous stasis. The organism tries to prevent the decreasing of the minute volume by the aid of compensatory mechanisms, such as hypertrophy of the heart muscle and tachycardia. Changes in the cardiac metabolism observed in the failing heart are partly derived from changes which cause the heart to fail, partly from the changes due to the compensatory mechanisms, which in turn partly contribute to the formation of incompensation.

The birth mechanism of heart failure is basically unknown in most cases, as is also the mechanism of therapeutic effects of cardiac glycosides.

Numerous findings have shown that ACh has a considerable effect on the function of the heart. Its significance as a mediator for a vagus stimulus was discovered by Loewi (1921). In addition, ACh has been shown to appear in the heart muscle tissue itself, and this ACh is considered essential to the rhythmic activity of the heart. Burn and co-workers have noticed that the functional activity of the rabbit auricles depends on the ACh synthesis that occurs in them (Burn and Vane 1949; Bülbring and Burn 1949).

The ACh metabolism of the heart in heart failure has not been studied extensively. Heart failure in the dog heart-lung preparation has been produced by inhibiting choline acetylase or ChE (Govier *et al.* 1953). Gremels (1937) considered that there was a cholinergic deficiency in heart failure. He noticed that the cardiac glycosides sensitized the heart to ACh. Similar observations have since been made by many others. Gremels thought that this perhaps derived from the ChE inhibiting effect of cardiac glycosides. This has been verified by Holland *et al.* (1954), for example.

We have not come across any reports of studies in the ACh metabolism of the heart in failure induced in a non-isolated heart. The isolated organ (the isolated heart or the heart-lung preparation) is not fully comparable with the heart which is in connection with the rest of the organism. Consequently, it was considered pertinent to study the ACh content and the ChE activities of the heart in cases of heart failure in the whole animal and also the effect of a cardiac glycoside upon these.

II. REVIEW OF THE LITERATURE

THE ACh CONTENT OF THE HEART

ACh has been detected in the heart of all species of animals investigated, the content varying considerably from one species to another. In mammals, the ACh content is greater in the auricles than in the ventricles (Engelhart 1930; Chang and Gaddum 1933); it is usually greater in the right auricle and ventricle than in the left.

In the rat auricles Rotschuh (1954 c) found the mean ACh to be 2.28 μg/g, in the right ventricle 1.30 μg/g and in the left 0.73 μg/g. Vlk (1958 b) observed 5.36 μg/g in the right auricle and 2.07 μg/g in the left. Chang and Gaddum (1933) reported that the average ACh content in the auricles of the dog was 1.9 μg/g and 0.07—0.35 μg/g in the ventricles, while according to Yokoyama (1955) it was 1.75 μg/g in the conducting tissue.

Abdon and Borglin (1945) obtained a figure of 0.21 μg/g for the ACh content of the whole rabbit heart, 0.38—0.46 μg/g for the content of the auricles and 0.12—0.135 μg/g for the ventricles, the last mentioned figure corresponding to the ACh content of the skeletal muscle. Vlk (1958 b) noted that the right auricle of the rabbit contained 1.37 μg/g, the left 0.78 μg/g. He considered the greater ACh content of the right auricle as being due to the greater concentration of nodal tissue in the right auricle and in the interauricular septum, which, when extracting, he attached to the right auricle.

As early as 1925, Witanovski concentrated on the fact that, when extracting, there was more ACh from the heart than was essential for the mediation of a vagus stimulus.

Abdon and co-workers showed that the ACh in the heart is mainly a protein-bound precursor from which a suitable stimulus

liberates ACh (Abdon and Hammarskjöld 1944; Abdon and Ljungdahl-Östberg 1944; Abdon 1945; Abdon and Borglin 1945).

Abdon and Borglin (1945) stated that a vagus stimulus decreases the ACh content of the rabbit heart considerably, although it does not disappear completely even after a prolonged stimulation. Vartiainen (1934), on the other hand, did not find that a vagus stimulus decreased the ACh content of the rabbit heart. Neither did Rotschuh and Berkel (1955) that of the rat or guinea-pig heart. The ACh content of the heart has been definitely found to depend on its vagus innervation. Before the development of the latter, no ACh is found in the dog embryo heart. In the auricles of 1—9 day-old dogs it was 1.47 μg/g; it was found only in small quantities in the ventricles. In the auricles of 4—5 month-old dogs it was 3.61 μg/g, in the ventricles 0.99 μg/g (Vlk 1958 a). On the other hand, many findings indicate that ACh is synthesized in the heart muscle tissue. ACh is liberated from the isolated heart without any vagus stimulus or by stimulating the heart directly to the auricles or ventricles (Rotschuh 1954 a). ACh is liberated from the isolated rabbit heart ventricles (Day 1956) in which vagal elements are questionable. ACh has also been found to exist in quite nerveless tissues, such as red cells, where it has been regarded as of importance to their permeability (Holland and Greig 1952) and in placenta (Bischoff *et al.* 1932).

THE ChE ACTIVITIES OF THE HEART

The mammal heart has been found to contain both types of ChE, specific and non-specific, though considerably more of the latter than the former. Briscoe (1954) noted MCh, a substrate of the specific ChE, dispersed more in the suspension made from the right auricle of the rabbit than that made from the left. The situation with BuCh, a substrate of the non-specific ChE, was quite the reverse. Both types of ChE appear less in the ventricles than in the auricles (Ord and Thompson 1950).

It has been found that the ChE of conductive tissue is, like the ChE of the peripheral nerves, specific and that in its activity it corresponds to the latter (Mommaerts *et al.* 1953). The view presented earlier that the ChE of the mammal heart is mainly non-specific, has been confirmed histochemically (Holmes 1957).

The development of the vagal innervation also affects the ChE activity. The ChE activity of the auricles of the guinea-pig reaches the values for a fully grown animal during the last few days before birth and that of the rabbit heart 10—12 days after birth (Shamarina 1941).

THE CARDIAC EFFECTS OF ACh AND THOSE OF ChE AND ITS INHIBITORS

When injecting ACh into whole animals the resulting cardiac effects are generally not purely cholinergic. In an atropinized animal the heart rate has been observed to increase (Feldberg and Minz 1932) as a result of a nicotine-like effect on the sympathetic ganglions. ACh has either a constricting or a dilating effect on the coronary vessels, depending on the experimental conditions. Hall (1939) reports having caused disturbances in the coronary circulation of the dog as well as congestive heart failure by repeatedly injecting great quantities of ACh intravenously. The negative inotropic and chronotropic effect of a vagus stimulus on the heart has been known since the last century; the corresponding effects of ACh were described by Dale (1914). Loewi's well-known experiment (1921) proved that the effects of the vagal stimulation are caused by liberated ACh.

The stimulating effects of ACh on the heart have also been described. In small concentrations (0.05—50 ng/ml) it has been found to stimulate the isolated cat, rabbit, and rat heart (McDowall 1946), as well as the guinea-pig heart (Spadolini 1948). This stimulating effect is stronger when the potassium content of the perfusion fluid is low (Graham 1949). On the other hand, it has been shown that atropine checks the stimulating effect of an electric stimulus on a Straub's heart stopped by increasing the potassium content (Giachetti 1951). ACh has caused an isolated auricular preparation of the rabbit heart to start beating again after stopping spontaneously (Bülbring and Burn 1949) or being stopped by proguanil (Burn and Vane 1949) or quinidine (Briscoe and Burn 1954). After the function of the auricles had returned to normal, the same quantities of ACh exercised an inhibiting effect. Bülbring and Burn (1949) discovered that the ACh synthesis of the auricles, when

they stopped, decreased down to a seventh of the original, and that it returned to almost normal when the function of the auricles became normal. A similar stimulating effect of ACh on the auricles has been observed when the temperature is reduced to 15—20°C (Burn and Rand 1957). Atropine checked this effect.

Apart from ACh, propionylcholine, butyrylcholine and carbacholine have been found to exercise a stimulating effect on the rat's auricles that have stopped spontaneously (Holtz and Westermann 1955).

Corresponding to the inhibitory effect of ACh, ChE has been seen to have a positive inotropic effect on the frog heart (Beznák 1958). The inhibitory effect of ChE has also been recorded (Spadolini and Giachetti 1941).

The cardiac effects of a vagus stimulus and ACh can be explained to a great extent by the changes caused by ACh in the permeability of the cell membrane; it especially increases the permeability to potassium and sodium (Holland *et al.* 1952; Burgen and Terroux 1953). According to Holland and co-workers (1952), this ion transport becomes maximal in conditions that are optimal for the activity of ChE. The direction of the ion transport depends on the prevailing intracellular and extracellular ion contents. To give an example, an increase in the extracellular potassium content reduces the escape of potassium from the cell and stops the auricular fibrillation caused by ACh (Burn, Gunning, and Walker 1956).

Changes in the permeability of the cellular membrane, again, cause changes in the bio-electric potentials. ACh has been shown to increase the conduction velocity in the cat auricle (Rijlant 1931), dog auricle (Hoffman *et al.* 1952), rabbit auricle (Vaughan Williams 1958) and frog ventricle (Rotschuh and Bammer 1952). ACh shortens the refractory period of the auricle (*e.g.* Hoffman, Siebens, and Brooks 1952). The effect of ACh on the level of the membrane potentials varies according to the prevailing test conditions. Obviously its effect is to raise the membrane resting potential, especially when the latter is unusually low. Burn and Rand (1957, 1958) believe that one of the functions of ACh is to maintain a sufficiently high resting potential, so that the pacemaker-impulses can depolarize adequately, and the excitation can spread. This mechanism, however, explains only a part of the cases in which ACh has a stimulating effect (Johnson and Robertson 1958).

It has been discovered that ACh prevents the slow depolarization during diastole (Brady and Hecht 1954; Hutter and Trautwein 1956). This has been explained as an inhibitory effect of a vagus stimulus on the ACh synthesis taking place in the sinoauricular node, and this would account for the decreased heart rate caused by the vagus stimulation. The slow depolarization would result from the increase of ACh concentration synthesized in the sinoauricular nodal tissue (Burn 1956). Vagus stimulus and ACh have been shown to retard the conduction of the excitation from the auricles to the ventricles (*e.g.* Gremels 1936; Hoffman *et al.* 1952).

While the auricular effect of ACh is considerable, its influence on the ventricles of the frog and mammal heart is much smaller (Dale 1930; Lanczos 1930). The same finding was made earlier with muscarine and pilocarpine (Cullis and Tribe 1913; Leetham 1913). ACh caused no changes in the bio-electric potentials in the ventricles of the mammal heart (Hoffman, Siebens and Brooks 1952; Hoffman and Suckling 1953). The last-mentioned believed that this resulted from the absence of nerve elements in the mammal ventricle. However, this difference between the auricle and ventricle appears regardless of nerve elements (Fänge *et al.* 1956).

The cardiac effects of ChE inhibitors are largely similar to those caused by ACh (Greene and Maneval 1932; Burn and Kottegoda 1953). In addition these substances have effects that cannot be explained by ChE inhibition. This is at least partly due to their competitive influence on ACh-receptors (Briscoe and Burn 1954). Burn and Walker made an interesting discovery in 1954. An inhibitor of the non-specific ChE (Nu 623) decreased the rate of the dog's heart, while an inhibitor of the specific ChE (284 C 51) did not.

In small quantities ACh has been found to have a "sparing effect" on the oxidative metabolism of the heart, while in great quantities it increases the oxygen consumption (Gremels 1936; Gollwitzer-Meier and Krüger 1938; Szekeres *et al.* 1958).

THE ACh METABOLISM OF THE HEART IN HEART FAILURE

Heart failure, the inability of the heart to perform its function as a pump properly, results from functional deficiency in one or both of the ventricles. In other words, the metabolic changes that

lead to heart failure are most likely to affect the ventricular muscle. On the other hand tachycardia, which is frequently encountered, and a tendency to fibrillation in heart failure point to disturbances in the formation of the excitation.

The insufficiency of the heart muscle may be thought to derive from a disorder in the formation of energy-rich phosphates or in their utilization, or from changes in contractile proteins (Friedberg 1956). In different cases there may be different factors; it has been observed clinically that the etiological factors behind heart failure vary to a considerable degree. Only in few types of heart failure can disturbances in the formation of the energy-rich phosphates be detected; more often the transformation of oxidative energy into muscular work has been affected (Wollenberger 1949). In most cases cardiac glycosides have been found to improve the energy utilization of the failing heart. It has also been proved that the actomyosin of the failing heart appears in a partly dissociated state (Benson 1955). The data of the amount of energy-rich phosphates in the failing heart is contradictory. It has been proved that their synthesis and utilization depend essentially on the ion milieu of a muscle cell. A decrease in intracellular potassium and an increase in sodium have been found to lower ATP-formation (Boyer *et al.* 1943; Utter 1950). The strength of the contraction of the myocardium has been proved to depend on the total quantities of potassium and sodium in the muscle cell (Szent-Györgyi 1956). A decrease in potassium and an increase in sodium have been noted in the failing human heart, while their total amount remains unchanged (Alexander *et al.* 1950; Clarke and Mosher 1952). Cardiac glycosides have a normalizing effect on the disturbed ion balance. So a possible defect in the mechanism regulating the extra-intracellular ion balance can have a weakening effect on the muscular contractility. The mechanism, by which the depolarization of the membrane of the muscle cell causes contraction, is not altogether known.

The hypothesis has been advanced that ACh plays some part in it (Abdon and Borglin 1945; Rotschuh 1954 a, c).

It has been ascertained that ACh activates ATP (Buchthal and Kahlson 1944). ACh does not itself cause a contraction of actomyosine (Torda and Wolff 1948). Beznák (1958) explained that the positive inotropic effect of ChE on the frog heart results from

the effect of ChE in increasing the concentration of essential purine derivatives and promoting their combination with contractile proteins.

Govier and co-workers (1953) produced heart failure in the heart-lung-preparation of the dog by inhibiting choline acetylase and ChE. Benzoylcholine and often g-strophanthin had a positive inotropic effect on failure caused by eserine and di-isopropyl-fluorophosphate (DFP). In failure caused by benzyl penicillin, benzoylcholine had a clear positive effect, while g-strophanthin had not. Benzyl penicillin has been proved to inhibit both ChE and choline acetylase. Govier and co-workers assumed that the positive inotropic effect of g-strophanthin derived at least partly from its limiting effect on ChE inhibition. They discovered that the ChE activity of the left ventricle increased after the administration of a cardiac glycoside.

Woske and co-workers (1953) studied the effect of the contraction strength of the heart on its excitability. They produced heart failure in the dog by compressing the thoracal aorta, but this did not change the excitability of the auricles or the ventricles. There were no changes in the action potential of the papillary muscle of the cat although its initial length and tension were changed (Trautwein and Dudel 1954). The tachycardia that occurs in heart failure has been considered to result from different mechanical, chemical, and neural stimuli, which decrease the tonus of the cardio-inhibitory centre and the vagus nerve and increase the tonus of the sympathetic system (Friedberg 1956). The heart rate of the rat has been observed to correlate inversely with its ACh content (Beznák 1956). On the other hand it has been proved that the excitability of the dog's heart muscle cells is not controlled by tonic vagus impulses, a bilateral vagotomy did not change the excitability of the auricles (Brooks *et al.* 1955). In the chicken embryo, in which the cardiac nervous system has not yet developed, the auricular and ventricular membrane potentials are similar to those of a full-grown animal qualitatively and quantitatively (Fingl, Woodbury and Hecht 1952). From the above it can obviously be concluded that in heart failure the factors regulating the heart rate mainly effect the pacemaker.

THE EFFECTS OF CARDIAC GLYCOSIDES ON THE ACh METABOLISM OF THE HEART AND ON THE CARDIAC EFFECTS OF ACh

Gremels (1937) considered heart failure to be at least partly due to the absence or weakening of the assimilator vagus effect. He noticed that small amounts of strophanthin strengthened the negative chronotropic effect of a vagus stimulus on the dog heart.

The inotropic and chronotropic cardiac glycoside effects are largely independent of one another (Rothlin and Bircher 1954). Therapeutic doses of cardiac glycosides can lower the heart rate, while the minute volume of the heart remains unchanged. On the other hand, the minute volume can increase while the rate remains unchanged. Atropine can turn the bradycardia caused by the glycosides to tachycardia, while the minute volume remains unaltered (Rothlin and Bircher 1954). Most investigators consider the bradycardia caused by cardiac glycosides as stemming on the one hand from a vagus stimulus and on the other from a direct effect on the heart muscle. Of these, only the former can be removed by atropine (Gold *et al.* 1939). The vagus effect of the cardiac glycosides has been explained in different ways: it has been regarded as a sensitization of a sinus caroticus-reflex (Heymans *et al.* 1932), an effect on the vagal centres (Greene and Peeler 1915; Korth *et al.* 1937), or a sensitization of the vagal ends in the sino-auricular node (Gremels 1935; Abdon and Nielsen 1938; Gold *et al.* 1939). It has been thought that small glycoside doses mainly provoke a vagus effect, and large doses a direct muscular effect (Gold *et al.* 1939). Atropine has been found to prevent the myocardial damage caused by toxic cardiac glycoside doses to some extent (Kyser *et al.* 1948). Lendle, Mercker and Rohr (1953) do not, however, regard the retardation of the heart rate by cardiac glycosides as due to a vagus stimulus but to an improvement in the contraction power of the heart itself.

Since Gremels (1937), numerous observations have been made of the sensitization of tissues by cardiac glycosides to the effect of ACh: the frog heart (Sedef 1951), the human foetal heart (Baker 1953), the rabbit heart (Perry and Reinert 1954; McEwen 1956), the rat heart (Benforado 1959), the skeletal muscle (Mazzella 1946), the superior sympathetic cervical ganglion (Konzett and Rothlin 1952;

Perry and Reinert 1954) and the neuromuscular conjunction (Westermann 1954). This sensitization is not specific to ACh, a corresponding sensitivity has also been observed to tetramethylammonium (Perry and Reinert 1954). Cardiac glycosides have been found to increase the effect of ACh in raising the blood pressure of an atropinized cat (Konzett and Carpi 1956). Not all, however, have been able to confirm this sensitizing effect (Luisada and Mautner 1946; Lendle and Oldenburg 1950; Rall *et al.* 1944). The different results may be due to different experimental circumstances. Sometimes a nicotine-like ACh effect has been foremost, sometimes a muscarine effect (Rothlin and Taeschler 1956).

It has been observed that ACh decreases the toxic effect of cardiac glycosides on the isolated heart (Chatterjee 1954; Holland, Greig and Dunn 1954). Large glycoside doses lower the potassium content of the heart muscle, and this may be prevented by ACh. The ChE activity, too, is then reduced and ACh increases it. On the other hand it was noticed that small glycoside doses preventing the loss of potassium from the heart muscle increase ACh hydrolysis (Holland, Greig and Dunn 1954). The initial stimulation of the tissue respiration caused by cardiac glycosides runs parallel to the size of the potassium transport and the ACh hydrolysis (Dunn, Holland and Greig 1954). The increase in ACh hydrolysis is connected with the reduction in the intracellular potassium content.

Gremels (1937) considered it possible that the sensitizing of the heart to ACh caused by cardiac glycosides derives from their inhibitory effect on ChE.

In vitro they have not been shown to clearly inhibit the specific or non-specific ChE (Kull 1939; Miquel and Riker 1945; Kimura 1949; Vincent 1947). On the other hand, cardiac glycosides have been found to inhibit ChE in vivo. Zinnitz and Rentz (1940) observed that k-strophanthin and digitoxin inhibited the serum ChE of the cat during retardation of the heart rate. Shinohara (1950) observed that lethal digitoxin, g-strophanthin and convallatoxin doses inhibited the ChE of the cat auricles 70—75 per cent and that of the ventricles by 73, 27 and 39 per cent respectively.

III. METHODS

EXPERIMENTAL HEART FAILURE

Heart failure can be divided clinically into the following groups (Friedberg 1956):

1. Left-sided heart failure.
2. Right-sided heart failure.
3. Combined left- and right-sided heart failure.
4. Heart failure associated with increased venous return.
5. Heart failure due to interference with inflow.

The object of experimental attempts to provoke heart failure has been to produce one of the cases mentioned above. The result has most often been more or less acute heart failure.

To produce heart failure a coronary artery has been ligated (Meesmann and Schmier 1955 b), or starch has been injected into the left ventricular cavity (Roos and Smith 1948). The myocardium has been damaged by cauterization (Starr *et al.* 1943) or by injecting toxic agents (Meesmann and Schmier 1955 a; Armstrong 1940). The pulmonary artery has been compressed (Fineberg and Wiggers 1936; Levy and Berne 1949), and in addition tricuspid insufficiency has been effected (Barger *et al.* 1950). Failure has also been caused by producing mitral stenosis or insufficiency (Hamilton *et al.* 1954), or aortic insufficiency (Dieckhoff 1936; Hawthorn *et al.* 1956), and by compressing the aorta (Alexander *et al.* 1953; Lindsey *et al.* 1957) or a kidney artery (Franco 1958). Large intravenous infusions have provoked an increase in the amount of circulating blood and heart failure (Huckabee *et al.* 1950; Gimbel *et al.* 1950). The same result has been achieved by operating arteriovenous shunts (Shipley *et al.* 1937; Bobb *et al.* 1952).

Alexander, Edmondson and Drury (1953) induced hypertonia and heart failure in the rabbit by compressing its abdominal aorta between the starting points of a. coeliaca and a. mesenterica cranialis. Immediately after the compression of the aorta, the animal's blood pressure showed an increase, which lasted for approximately half an hour, and afterwards fell to normal and even below. For 1—6 days after the operation the blood pressure went up again and stayed high until the death of the animal. On the day following the operation the rabbit's appetite was poor, but reverted to almost normal during the following days. Subsequently the animal, which now looked healthy, began to eat and drink less, respiration became heavy, and symptoms of orthopnea and cyanosis could be detected. The rabbits then died in a few hours to days following the onset of the symptoms. Two thirds of all the rabbits operated upon by Alexander and co-workers died within 2 weeks, most of them during the week following the operation. At post-mortem typical stasis changes could be detected: lung and liver congestion, in most cases fluid in the pleural or peritoneal cavity, in some cases subcutaneous edema also. The heart was dilated and hypertrophied. The kidneys showed only slight signs of passive congestion. Signs of capillary congestion, edema fluid in the alveoli, and alveolar hemorrhages could be detected in the lungs microscopically, as well as thickening of the septa of the alveoli. Cells typical of heart failure were discovered in the animals that survived for a month or more after the operation. Rabbits that died within five days after the operation showed the clearest signs of pulmonary edema and alveolar hemorrhages. In the liver signs of passive congestion were detectable during the first week.

COMPRESSION OF THE AORTA AND DETECTION OF HEART FAILURE

The rabbit was kept without food overnight. The laparotomy was made under ether anesthesia, and the aorta revealed between the origins of a. coeliaca and a. mesenterica cranialis. An artery clamp made of aluminium or silver (size $5 \times 5 \times 8$ mm.) was attached to it. The screw was tightened, until the animal's respiration grew clearly laboured, after which the screw was slackened

¼—½ turns (0.1—0.2 mm). The compression of the aorta was generally found sufficient when the space between the base and the screw of the clamp was 0.7—1.2 mm, and the cross-section area of the aorta had shrunk to one fourth of its original size. Often a rubber piece 1 mm in thickness was placed between the screw and the aorta, to produce a more even pressure, and to prevent traumatisation. Heart failure was confirmed in approximately 60 per cent of the operated rabbits. The others died either immediately after operation, probably from the aorta having been compressed too tightly, or later from infection. In some cases no increase in blood pressure and no heart failure could be detected.

Approximately one week prior to compression of the aorta the rabbit was placed for observation in a mesh cage with a detachable bottom. The animal's blood pressure was registered and the amount of water and nourishment (oats and swedes) consumed were measured daily. To record the heart rate, an electrocardiogram was taken before compressing the aorta and killing the animal, and, in cases where the rabbits had been treated with strophanthin, before its injection. The rabbits were weighed every other day, or more often, to an accuracy of ± 10 g. Observation was continued in the same way after compressing the aorta.

The symptoms listed by Alexander, Edmondson and Drury (1953) served as the criteria for heart failure: difficulty in breathing and orthopnea, cyanosis and reduced appetite. Heart failure was verified at the post-mortem examination macroscopically and microscopically.

The animals were killed by a blow on the neck and by severing the carotid arteries. Possible occurrence of subcutaneous edema was watched for, and the fluid in the pleural or peritoneal cavity was measured. The heart, lungs and liver were weighed, and the macroscopic signs of congestion in the lungs and the liver were identified. Samples of these organs were taken for microscopic examination. Formalin-fixed material was embedded in paraffin and stained using Weigert's hematoxylin-van Gieson stain.

MEASURING THE BLOOD PRESSURE

There are several methods for measuring a rabbit's blood pressure. Of these, van Leersum's (1911) has proved reliable.

Under ether anesthesia two parallel ventral incisions of appr. 5 cm were made lengthwise in the neck about 1 cm apart. The skin of the strip was detached from its base, the left common carotid artery was separated and placed in the strip. The edges were sewn together and the incision under the loop was closed. A bandage containing neomycin-bacitracin ointment (Ungt. Bacimycin, Orion) was wrapped around the loop to prevent drying and infection.

A flat cuff made of rubber and cloth (size 5 $\times$ 1 cm) connected to a mercury manometer, was used to measure the blood pressure. It was measured palpatorically to an accuracy of $\pm$ 5 mmHg. The measuring was continued until equal values were reached. Another widely used method for measuring the blood pressure is Grant and Rothchild's (1934) ear capsule method. Because of the reactivity of the animal, this method gives reliable results only under absolutely constant conditions. Although the values obtained from the carotid loop are appr. 15—20 mmHg higher than those obtained directly from the common carotid artery, according to Schlesinger's (1930) and my own experience they are equal and comparable to one another.

REGISTERING THE HEART RATE

The heart rate was registered electrocardiographically with a "Mingograf 12" apparatus (Elema AB.), the paper speed was 50 mm/sec. To eliminate external disturbances the rabbit was placed in a cage made of metal mesh during the time of recording. The rabbits were allowed to take up typical sitting position as in the experiments of Slapak and Hermanek (1951), since it has been observed that stretching the legs to the base can cause damage to the heart muscle (Veith 1940). In accordance with the method described by Massmann and Opitz (1954), needle electrodes were fastened under the skin to the extremities. When the rabbit became tranquil and its breathing even, recording was started, the heart rate being counted from the standard limb connections.

TREATMENT OF RABBITS WITH STROPHANTHIN

According to Nyiri and DuBois (1930), the mean lethal g-strophanthin dose for the rabbit, as reckoned by Hatcher's (1912) method is 0.183 mg/kg, the range being 0.114—0.244 mg/kg. Lendle (1933) considered the mean lethal dose to be 0.16 mg/kg when using constant infusion, the variation being greater than with Hatcher's method. It is difficult to determine the lethal strophanthin dose in the rabbit because smaller doses often cause respiratory paralysis. Using Hatcher's method, Lendle obtained lethal doses of 0.16—0.18 mg/kg. Rabbits can eliminate cardiac glycosides better than most other mammals. Hauptstein (1927) found that tolerance increased in a regular manner when the infusion of g-strophanthin was slowed down and the infusion period extended, from which he calculated that rabbits can eliminate 0.02 mg/kg/hr. According to Hauptstein and Lendle (1933), the rate of elimination can even be 0.03 mg/kg/hr., the corresponding figure for the cat, for example, being only 0.006 mg/kg/hr. The g-strophanthin (Merck & Co.) doses used in the present work and the times chosen for their injection were determined according to the doses used as medication for rabbits with heart failure. Doses of 0.02—0.03 mg/kg were found to relieve laboured respiration in these; a new dose was injected whenever the animal's respiration became laboured. Doses of this size seemed suitable, partly because they facilitated respiration and partly because of the bradycardic effect, which is one criterion for favourable glycoside effects in heart failure. Each time g-strophanthin was injected into the vein of the ear for 5 minutes.

TAKING SPECIMENS FOR THE DETERMINATION OF ACh AND ChE

On account of the relative lability of ACh the rabbits had to be killed. Of the rabbits that died spontaneously, only those that had been dead for two hours at the most were included in the test series. Rotschuh (1954 b) examined the ACh stability in the frog heart kept in Ringer's solution at 20—22°C. During the first two hours after the animal's death only a slight decline occurred. Sub-

sequently it fell off sharply. The ChE stability is fairly good, as Keiling and Wang (1947), for example, have shown. Before weighing the heart, the ventricles and auricles were opened, the blood was washed away as throughly as possible with saline after which the heart was dried with filter paper. The samples for the ACh determinations were taken first and extracted immediately. Samples for the determination of the ChE activities were then taken and kept at —18°C until they could be assayed. The interauricular septum was attached to the right auricle, as done by Briscoe (1954) and Vlk (1958 b). From the interventricular septum the same part, the basal half, was taken each time.

EXAMINATION OF THE ACh CONTENT OF THE HEART

EXTRACTION OF ACh FROM THE HEART MUSCLE

To extract ACh, Witanowski (1925) used alcohol, Galehr and Plattner (1928), Chang and Gaddum (1933) and Beznák (1934) trichloracetic acid, Engelhart (1930) hydrochloric acid-alcohol, and Bischoff *et al.* (1931) metaphosphoric acid. Loewi and Hellauer (1938) discovered that the ACh yield was greater when extracted with trichloracetic acid than when extracted with HCl-alcohol, and that the quantity increased when the extract was heated in a boiling water-bath for 5 minutes.

Abdon and Hammarskjöld (1944) proved that ACh exists in the heart as a precursor, in a bound form. In an acid milieu ACh can be liberated by heating. According to Abdon and Ljungdahl-Östberg (1944), this precursor dissolves in alcohol, but not in acetone, as free ACh and choline do. According to Prajmovsky and Welsh (1948), the ACh yield is the same whether the extract acidified with hydrochloric acid is allowed to stand for an hour at room temperature, is heated for a few minutes at 70°C, or is boiled in a neutral solution for 3 minutes.

Abdon and Hammarskjöld (1944) froze the heart with solid carbon dioxide or liquid air. Rotschuh (1954 b) did not detect any essential difference in the ACh yield from the frozen heart and from the heart prepared at room temperature.

In the present work ACh was extracted using the method described by Rotschuh (1954 b). The composition of the Ringer's

solution employed was NaCl 0.6, KCl 0.0075 and $CaCl_2$ 0.01 per cent. This was acidified with hydrochloric acid (0.1 n) to pH 4. According to Abdon and Ljungdahl-Östberg (1944), ACh, like its precursor, is most stable at pH 4. Eserine was added in concentration 10^{-5}. The sample (100– 300 mg cardiac tissue) was placed in 3 ml of this solution. The muscle was scissored into as small pieces as possible. Rotschuh (1954 b) compared the ACh yield of samples cut by scissors and of those ground in a mortar with sand and found them equal. It was boiled in a water-bath for 5 min., centrifuged and refrigerated until the samples could be assayed, for a maximum of 48 hours. Immediately before assay, the samples were neutralized with 0.1 n sodium hydroxide. The neutrality was determined with bromthymol blue and indicator paper. During the assay the extracts as well as the control solution (Ringer's solution of ACh) were kept in ice water to prevent hydrolysis.

DETERMINATION OF ACh

In the biological assay of ACh two main methods have commonly been used, the dorsal muscle of the leech and the rectus abdominis muscle of the frog. Although the latter is more exact, the sensitivity is considerably less. Recently Rotschuh (1954 b) has used the rectus muscle of the frog sensitized with diethyl-p-nitrophenyl-phosphate (Mintacol) and with it has been able to determine an ACh content ten times smaller than without sensitization. Nevertheless, for the assay in the present work, the rectus muscle has not proved sufficiently sensitive despite such sensitization. Consequently ACh has been determined using the dorsal muscle of the leech (Hirudo medicinalis). Its advantage is its great specificity and sensitivity. The disadvantage is the often rather large method error (20 per cent) and the fact that the preparation requires a long relaxation period (Minz 1932).

The dorsal muscle of the leech was prepared using Minz's method (1932). The composition of the Ringer's solution was NaCl 0.6, KCl 0.0075, $NaHCO_3$ and $CaCl_2$, both 0.01 per cent. Contrary to the experiences with the rectus muscle of the frog, only greater potassium concentrations are likely to contract it after an initial relaxation (Gollwitzer-Meier and Krüger 1934; Vartiainen and Kostia 1937).

To sensitize the preparation to ACh, eserine in dilution 10^{-5} was added to the Ringer's solution. This eserine concentration also proved useful in the experiments conducted by Viikari (1955). The muscle was suspended in 2 ml of the Ringer's solution described above, and a slow current of oxygen was passed through the solution. The eserine was allowed to sensitize for 15 min. before the test solution was added, which, again, was allowed to affect the preparation for two minutes. After that it was rinsed several times with Ringer's solution without eserine. The interval between the application of two successive test solutions was 30 min. The minimum quantity of ACh that could be determined was usually 5—10 ng.

Figure 1 shows the response of the dorsal muscle of a leech to various amounts of ACh.

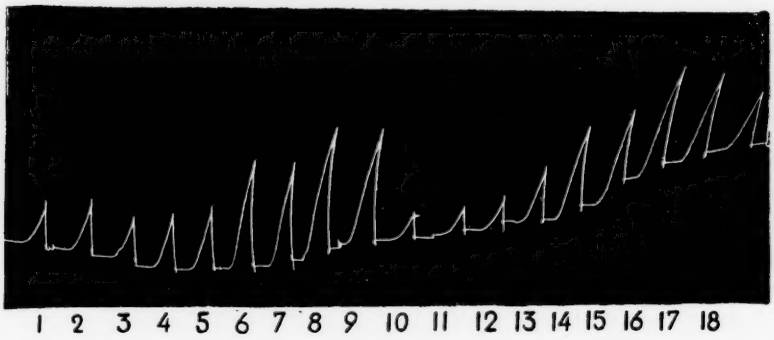

Fig. 1. — Responses of the dorsal muscle of the leech to various amounts of ACh. 1 = 2 ng, 3 and 12 = 3 ng, 7 and 18 = 10 ng, 8 and 14 = 15 ng, 16 = 20 ng. The others show responses to test samples.

DETERMINATION OF THE ChE ACTIVITIES OF THE HEART

GASOMETRIC METHOD

The determination of the ChE activity is based upon measuring the hydrolysis of ACh or other substrate in a reaction mixture. It can be done by determining the residual substrate biologically or colorimetrically, or by determining the organic acid liberated in hydrolysis by titration or potentiometrically. The gasometric method is based on the manometric measurement of the amount of carbon dioxide that develops in a system containing bicarbonate

due to the acetic acid discharged in hydrolysis. The method is fairly fast and is good for serial experiments. In the present work it was used according to Augustinsson's (1948) method, modified for the prevailing present test conditions. A standard Warburg-apparatus was employed. Conical bottles, c. 5.5 ml in volume, with a side-bulb were used as reaction vessels. These were calibrated with mercury.

The manometric fluid used was Brodie's solution (Umbreit *et al.* 1951). The volume of the reaction mixture was always 0.40 ml. The main part of the bottle contained 0.30 ml of tissue suspension, and the side bulb 0.10 ml of substrate solution (0.12 M). The substrate was dissolved and an enzyme suspension was made in bicarbonate buffer solution (Augustinsson's R_{30}), the composition of which is given in Table 1.

TABLE 1

THE COMPOSITION OF BICARBONATE-RINGER'S SOLUTION

Solution	Per cent (w/v)	ml	R_{30} molarity
NaCl	0.90	100	1.15×10^{-1}
$NaHCO_3$	1.26	30	3.36×10^{-2}
KCl	1.20	2	2.40×10^{-3}
$CaCl_2 \cdot 6H_2O$	1.76	2	1.20×10^{-3}

The pH of the solution saturated with gas mixture (95 vol. per cent N_2 and 5 vol.per cent CO_2) was 7.40. This was controlled by using a glass electrode or indicator paper.

Hydrolysis was conducted in the reaction mixture saturated with the gas mixture described earlier. Before mixing the tissue suspension and substrate solution, the manometers with their reaction vessels were shaken in a thermostat (37.5°C) for 15 min. The manometers were read at one minute intervals and lifted from their stands at zero time. The contents of the reaction vessels were mixed, and the side bulbs were rinsed with the whole mixture. During the assay each manometer was read at 10 min. intervals. In the results the values are given for 60 min. Each time two manometers were used to measure the spontaneous hydrolysis, while one manometer (containing 0.4 ml aq.dest.) was used as a thermobarometer.

ENZYME PREPARATION

The tissue samples preserved at —18°C were ground in a mortar with sand. The final tissue dilution in the reaction mixture was 1:10. Because of the small quantities of tissue available the whole tissue suspension was used.

SUBSTRATES

The substrates used were acetylcholine chloride (Hoffmann — La Roche & Co.), d, l-acetyl-β-methylcholine chloride (Light & Co.) and butyrylcholine chloride (Light & Co.). Govier *et al.* (1953) used benzoylcholine as a substrate when determining the non-specific cholinesterase. Mendel and Myers (1956) and others preferred BuCh. The final substrate concentration of all the substrates was 0.03 M.

THE ACCURACY OF THE METHOD

The same tissue suspension (right ventricle) was used in 6 parallel tests, the substrate being BuCh. The readings were 34.8, 37.8, 38.3, 38.7, 39.1 and 40.3. The average was 38.2 $\pm$ 0.76 ($\pm$ the standard error of the mean) and the standard deviation 1.85.

STATISTICAL TREATMENT

The material has been tested throughout by means of the Wilcoxon test, which is based on the mutual order of magnitude of the numbers of two series. The results are represented by denoting the random probability P, which indicates the maximum probability for the difference in magnitude observed between two averages being purely random. If P exceeds the value 0.05, the difference has been considered statistically non-significant.

IV. RESULTS

IN VIVO FINDINGS IN RABBITS AND POST-MORTEM MACROSCOPIC AND MICROSCOPIC CHANGES

The distribution of the material used in this work into different test groups and the assays made are presented in Table 2.

TABLE 2

DISTRIBUTION OF RABBIT MATERIAL AND ACh AND ChE DETERMINATIONS MADE

Rabbit group	Number of animals	Rabbit No.	Assays			
			ACh	ChE		
				Substrates		
				MCh	BuCh	ACh
Untreated control rabbits (CR)	12	1		+		
		2		+	+	+
		3		+	+	+
		4		+	+	+
		5	+	+	+	
		6	+		+	+
		7	+	+		+
		8	+		+	
		9	+			+
		10	+		+	
		11			+	
		12			+	
Control rabbits treated with small doses of strophanthin (SCR)	4	1	+		+	
		2	+		+	
		3	+		+	
		4	+		+	
Control rabbits treated with larger doses of strophanthin (SCR)	4	5	+		+	
		6	+		+	
		7	+		+	
		8	+		+	

Continued

Rabbit group	Number of animals	Rabbit No.	Assays			
			ACh	ChE		
				Substrates		
				MCh	BuCh	ACh
Rabbits with heart failure that survived less than 2 days after compression of the aorta (IR)	6	1			+	
		2			+	
		3	+		+	
		4			+	
		5			+	
		6			+	
Rabbits with heart failure that survived 2 or more days after compression of the aorta (IR)	15	7	+		+	
		8	+	+	+	+
		9	+	+	+	
		10	+		+	
		11	+		+	
		12		+	+	
		13		+	+	+
		14		+	+	+
		15	+			
		16	+		+	
		17		+	+	+
		18	+		+	+
		19	+		+	
		20	+	+	+	+
		21	+		+	
Hypertonic rabbits in which there were no symptoms of heart failure in vivo but signs of congestion at post-mortem (IR)	3	22	+		+	
		23	+		+	
		24	+		+	
Rabbits with heart failure treated with strophanthin (SIR)	6	1	+		+	
		2	+		+	
		3	+		+	
		4	+		+	
		5	+		+	
		6	+		+	

UNTREATED CONTROL RABBITS

The sex, weight, weight of the heart and lungs, and the percentage of these of the total weight, are shown in Table 3.

TABLE 3

CONTROL RABBITS

Sex, weight of animal, weight of heart and lungs, percentage of these of whole weight.

CR	Sex	Weight g	Heart		Lungs	
			g	per cent	g	per cent
1	♂	2 060	4.95	0.24	6.9	0.33
2	♂	3 050	5.21	0.17	8.1	0.27
3	♂	1 470	2.94	0.20	3.9	0.27
4	♀	1 720	3.25	0.19	5.7	0.33
5	♀	2 200	4.46	0.20	9.0	0.41
6	♀	2 460	4.47	0.18	8.9	0.36
7	♀	2 430	6.25	0.26		
8	♀	3 020	6.92	0.23	9.9	0.33
9	♀	1 950	5.50	0.28	8.2	0.42
10	♂	2 010	5.65	0.28	8.9	0.44
11	♂	2 100	5.05	0.24	7.8	0.37
12	♂	3 460	6.25	0.18	11.6	0.34
mean		2 327	5.07	0.22	8.1	0.35
range		1 470— 3 460	2.94— 6.92	0.17— 0.28	3.9— 11.6	0.27— 0.44

STROPHANTHIN-TREATED CONTROL RABBITS

The sex and weight of the rabbits treated with g-strophanthin, their heart weight and its percentage of the body weight, and the strophanthin doses administered are shown in Table 4.

Post-mortem examination showed that a strophanthin injection in therapeutic doses did not produce any signs differing from those of normal rabbits. The changes in the heart rate are shown in Table 5. A deceleration appears in only two of the four rabbits thus treated.

Table 5 also shows the corresponding data for rabbits treated with greater doses of g-strophanthin; a toxic glycoside effect was aimed at.

The strophanthin doses administered to these rabbits are thus 2—5 times greater than those given to the former group. The difference in the dosage is due to the fact that toxic symptoms appeared in different rabbits with different amounts of strophanthin.

In rabbit SCR 5, laboured and rapid breathing, motor restless-

TABLE 4

CONTROL RABBITS TREATED WITH STROPHANTHIN

Sex, weight, heart weight, percentage of whole weight, and strophanthin treatment given.

SCR	Sex	Weight g	Heart		Strophanthin treatment
			g	per cent	
1	♂	3 560	7.80	0.22	0.02 mg/kg three times (7 and 16 hr. intervals). Killed 4 hrs. after the last dose.
2	♀	3 170	7.10	0.22	0.02 mg/kg twice (6 hr. interval). Killed 4 hrs. after the last dose.
3	♀	2 490	5.89	0.24	0.02 mg/kg three times (6 and 16 hr. intervals). Killed 6 hrs. after the last dose.
4	♀	2 200	5.58	0.25	0.02 mg/kg twice (17 hr. interval). Killed 9 hrs. after the last dose.
mean		2 855	6.59	0.23	
range		2 200— 3 560	5.58— 7.80	0.22— 0.25	
5	♀	4 200	7.69	0.18	0.05 mg/kg twice (½ hr. interval). Killed 1 ½ hrs. after the last dose
6	♀	2 580	6.98	0.27	0.05 mg/kg twice (½hr. interval). Killed 5 ½ hrs. after the last dose.
7	♂	2 460	5.73	0.23	First given 0.10 mg/kg and after 4 hr. 0.05 mg/kg. Killed 3 ½ hrs. after the last dose.
8	♂	3 170	7.35	0.23	First given 0.10 mg/kg and after 3 ½ hr. 0.05 mg/kg. Killed 2 ½ hrs. after the last dose.
mean		3 103	6.94	0.23	
range		2 460— 4 200	5.73— 7.69	0.18— 0.27	

ness and convulsions appeared. In the electrocardiogram (ECG) heterotopic ventricular extrasystoles occurred. In rabbit SCR 6 breathing was laboured and in the ECG the T-waves were low. Similar symptoms were found in rabbit SCR 7. Rabbit SCR 8 showed the aforementioned symptoms and also a broadening of the QRS wave in the ECG. According to Table 5, an increase in heart rate appeared in three of these four rabbits and a decrease in one.

In all the control rabbits treated with larger doses of strophanthin, lung edema was found at the post-mortem examination.

TABLE 5

THE HEART RATE OF CONTROL RABBITS TREATED WITH STROPHANTHIN

Heart rate before strophanthin administration and before killing the rabbit and the percentual change in the rates after strophanthin.

SCR	Rate before stroph./min.	Rate before killing/min.	Change in rate per cent
1	250	270	+ 8.0
2	280	220	—21.4
3	235	235	0
4	280	225	—19.6
5	240	250	+ 4.2
6	250	205	—18.0
7	205	235	+14.6
8	235	260	+10.6

UNTREATED RABBITS WITH HEART FAILURE

These have been divided into three groups. Six of them died within the first day after compression of the aorta. Symptoms of heart failure were found in all in vivo and at post-mortem. Showing acute failure they corresponded best to many of the experimental heart failures described in the literature, and have therefore been formed into a group of their own. Further, they all died spontaneously. Despite an observation period of 19, 20 and 83 days, no symptoms of heart failure were found in three rabbits (IR 22—24) in vivo, although clear hypertonia developed in all of them. Because congestive signs in the lungs and liver were discerned in all of them they have been classified as rabbits with heart failure. Perhaps during a longer observation period in vivo symptoms of heart failure would have developed. The other rabbits in the group survived for 2—40 days after the compression of the aorta.

Table 6 gives the following data on the rabbits that survived less than two days after the compression of the aorta and in which there were symptoms of heart failure: sex, weight from commencement of observation in cage, observation period before compression of the aorta, weight at operation, the percentual change in body weight, and the survival period after compression of the aorta. In none of these rabbits did the weight change essentially after operation. In rabbit IR 3 the blood pressure was observed to have

TABLE 6

IN VIVO-FINDINGS OF RABBITS WITH HEART FAILURE THAT SURVIVED LESS THAN TWO DAYS

Sex, weight at beginning of observation, observation period before compression of the aorta, weight at operation, percentual change in weight, survival time after operation.

IR	Sex	Weight at beginning of observ. days	Observ. time days	Weight at oper. g	Change in weight per cent	Survival time after oper. h.
1	♂	2 480	6	2 450	— 1.2	6
2	♀	2 100	5	2 140	+ 1.9	6
3	♂	2 220	6	2 370	+ 7.2	8
4	♀	2 830	6	3 210	+13.4	23
5	♀	1 650	7	1 580	— 4.2	24
6	♂	3 220	6	3 250	+ 0.9	24
mean		2 417		2 500	+ 3.0	15
range		1 650— 3 220		1 580— 3 250	— 4.2— +13.4	6—24

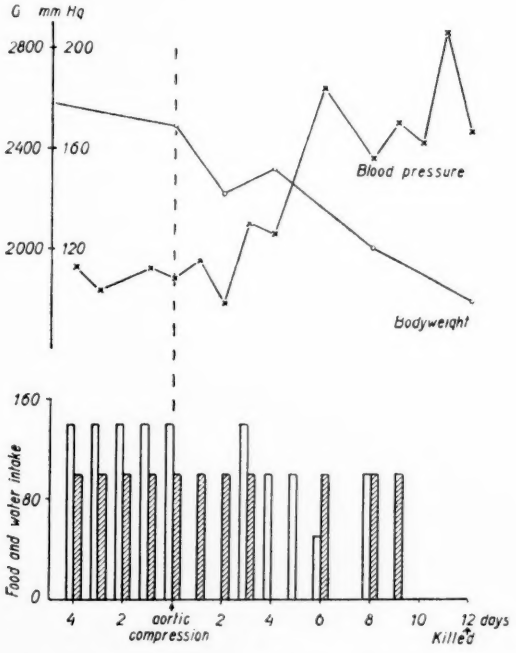

Fig. 2. — Changes in blood pressure and body weight, and the food and water intake of a rabbit with heart failure (IR 18) before and after aortic compression.

In the upper scale, the body weight g and blood pressure mmHg as ordinate, in the lower scale, the food and water intake g. White columns = food, shaded columns = water. Time in days as abscissa.

TABLE 7

POST-MORTEM FINDINGS IN RABBITS WITH HEART FAILURE THAT SURVIVED LESS THAN TWO DAYS

Heart and lung weights, their percentage of whole weight, macroscopically and microscopically observed congestion and the appearance of fluid in the pleural and peritoneal cavities and subcutaneously.

IR	Heart		Lungs		Macr. obs.	Micr. obs.	Liver		Fluid		
							macr. obs.	micr. obs.			
	g	per cent	g	per cent					pleural	perit. ml	subcut.
1	4.41	0.18	7.3	0.30	C	C	0	0	0	0	0
2	4.83	0.23			C	C	C		0	0	0
3	7.21	0.30	11.8	0.50	C	C	C	C	0	0	0
4	9.40	0.29	23.3	0.73	C	C	C	C	0	+++	+
5	2.72	0.17	7.0	0.44	E	E	C	C	20	0	0
6	8.60	0.26			C	C	C		0	+	0
Mean	6.19	0.24	12.3	0.49							
Range	2.72— 9.40	0.17 0.30	7.0— 23.3	0.30— 0.73							

C = congestion, E = edema, 0 = no difference from normal.

Because it has not been possible to measure the amount of ascites fluid accurately, it has been marked: + =under 10 ml, ++=10—50 ml, +++ = over 50 ml.

increased from the pre-operation value of 115—130 to 145. All the rabbits died spontaneously.

In the lungs of all the rabbits an increase in bloodiness or edema was found at the post-mortem examination. There was liver congestion in rabbits 2—6. The weights of the heart and lungs, their percentage of the total weight, the appearance of fluid in the pleural or peritoneal cavities, and of subcutaneous edema and microscopically discernible congestion in the lungs and liver are shown in Table 7.

No significant increase in the weight of the liver was observed in any of the groups of animals operated upon.

Table 8 gives the sex, changes in weight and blood pressure, and manner of death of rabbits with heart failure that survived for 2—40 days.

In all these rabbits a decrease in weight appears after operation. This may be due to a deterioration of appetite. Before the aorta was compressed a slight fall in weight occurred in few rabbits only. The blood pressure showed a rise in all cases in which

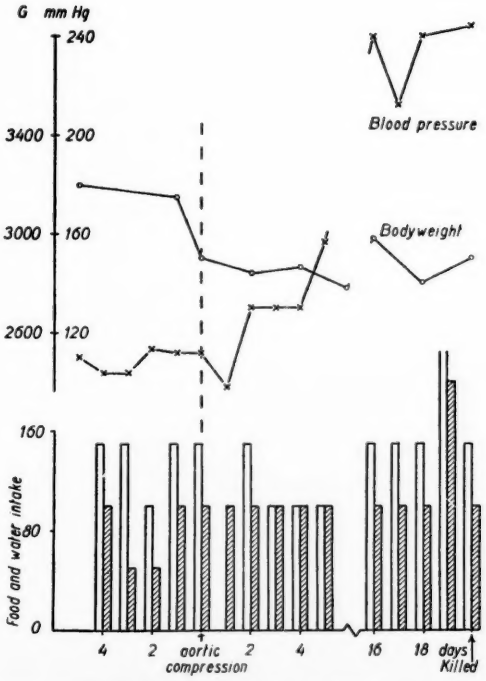

Fig. 3. — The same observations as in Figure 2 for a rabbit (IR 23) which did not show symptoms of heart failure in vivo.

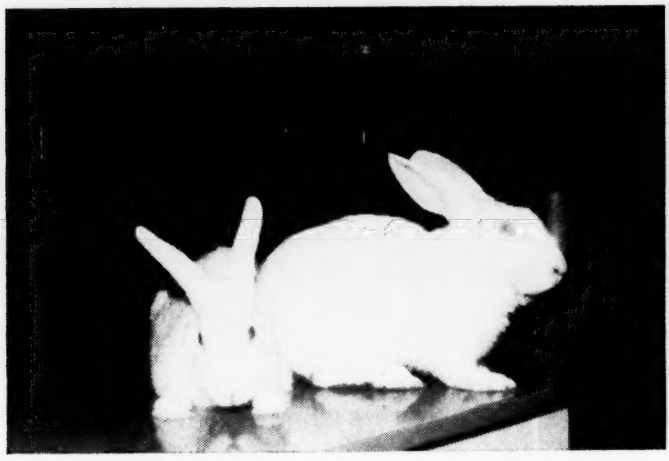

Fig. 4. — Typical sitting positions of a healthy rabbit (left) and of a rabbit in which heart failure has been detected (right).

TABLE 8

IN VIVO FINDINGS OF RABBITS WITH HEART FAILURE THAT SURVIVED 2—83 DAYS

Sex, weight at beginning of observation, observation period before compression of the aorta, weight at operation, change in weight, survival time after operation, weight at death, change from operation weight, blood pressure before operation, maximum blood pressure measured, blood pressure before death, manner of death (K = killed, S = succumbed).

IR	Sex	Weight at beginning of observation g	Observ. time days	Weight at operation g	Change in weight per cent	Survival time days	Weight at death g	Change in weight per cent	Blood pressure before oper.	Blood pressure max. mmHg	Blood pressure before death	Manner of death
7	♀	2 100	7	2 280	+ 8.6	2	2 030	—10.9	110—120	140	140	S
8	♀	2 250	4	2 380	+ 6.7	2	1 910	—19.8	120—130	160	160	S
9	♀	2 140	8	2 100	— 1.9	3	1 850	—11.9	90—110	140	140	K
10	♀	2 600	8	2 590	— 0.4	3	2 480	— 4.3	110—120	155	110	K
11	♂	2 370	8	2 460	+ 3.8	3	2 070	—15.8	105—120	140	140	K
12	♀	2 260	6	2 260	0	4	1 960	—13.6				K
13	♀	2 850	6	2 910	+ 2.1	4	2 850	— 2.1	120—130	180	155	K
14	♀	3 780	4	3 850	+ 1.8	5	3 240	—16.3				K
15	♂	2 400	8	2 380	— 0.8	6	2 190	— 8.0	120—130	150	150	K
16	♂	2 780	8	2 780	0	8	2 280	—18.0	125—130	195	175	K
17	♀	1 640	8	1 930	+17.7	8	1 790	— 7.2				S
18	♂	2 580	8	2 490	— 3.5	12	1 790	—28.1	105—110	205	165	K
19	♂	2 700	7	2 710	+ 0.4	12	2 330	—14.0				K
20	♀	1 670	6	1 725	+ 3.3	39	1 500	—13.1				S
21	♂	2 980	7	2 900	— 2.7	40	2 740	— 5.5	100—120	195	175	K
Mean		2 473	7	2 516	+ 2.3	10	2 201	—12.1	116	166	151	
Range		1 640—3 780	4—8	1 725—3 850	— 3.3—+17.7	2—40	1 500—3 240	— 2.1——28.1	90—130	140—205	110—175	
22	♂	2 510	8	2 550	+ 1.6	19	2 450	— 3.9	110—120	240	195	K
23	♀	3 290	8	2 900	— 8.8	20	2 900	0	105—115	245	245	K
24	♀	2 230	8	2 180	— 2.2	83	2 400	+10.1	110—130	230	180	K
Mean		2 677	8	2 543	— 3.1		2 583	+ 2.1	116	238	207	

TABLE 9

THE HEART RATE OF RABBITS WITH HEART FAILURE

Heart rate before compression of the aorta and before strophanthin administration, and percentual change. Rate before killing rabbits and change from that observed before strophanthin administration.

Animal		Rate before operation /min.	Rate before strophanthin /min.	Change per cent	Rate before killing /min.	Change per cent
	9	210			122	—41.9
IR	10				280	
	11				215	
	13	240			250	+ 4.2
	16				245	
	18	175			240	+37.1
	19	235			235	0
	20	265			270	+ 1.9
	21				265	
	2	240	270	+12.5	265	— 1.9
SIR	3		275		240	—12.7
	4	255	250	— 1.9	165	—34.0
	5	270	275	+ 1.9	225	—18.2
	6	240	250	+ 4.2	200	—20.0

it was measured. Picture 2 shows the changes in blood pressure and weight in a rabbit (IR 18) in this group and the animal's food and water intake before operation and after. For the sake of comparison, Fig. 3 shows the corresponding findings in a rabbit in which hypertonia developed but no heart failure was found in vivo (IR 23). Here the weight at death was roughly the same as at operation, and the appetite remained good up till the time the animal was killed.

Fig. 4 shows a normal rabbit in a typical resting position and a rabbit in which heart failure has been detected. The head is upright — a sign of orthopnea.

The heart rates of rabbits with heart failure treated with strophanthin and untreated ones are presented in Table 9.

In the untreated rabbits with heart failure, great variation can be see in the rate, the change from the pre-operation rate ranging from —41.9 to + 37.1 per cent.

Table 10 shows the changes observed after death. There were signs of congestion in the lungs and liver of all the rabbits. In many there was fluid in the pleural and peritoneal cavities, in three cases subcutaneous edema.

TABLE 10

POST-MORTEM FINDINGS IN RABBITS WITH HEART FAILURE THAT SURVIVED 2—83 DAYS

Heart and lung weights, their percentage of whole weight, macroscopically and microscopically observed congestion and the appearance of fluid in the pleural and peritoneal cavities and subcutaneously.

IR	Heart		Lungs				Liver		Fluid		
	g	per cent	g	per cent	Macr. obs.	Micr. obs.	macr. obs.	micr. obs.	pleur.	perit. ml	subcut.
7	4.44	0.22	8.4	0.41	C	C	C	C	0	0	0
8	6.11	0.32	15.3	0.80	E	E	C	C	5	++	+
9	4.57	0.25	13.5	0.73	E	E	C	C	10	++	0
10	7.62	0.31	10.5	0.42	E	E	C	C	5	+	+
11	5.96	0.29	8.9	0.43	E	E	C	C	0	0	0
12	5.82	0.30	13.5	0.69	C	C	C	C	5	++	0
13	6.88	0.24	11.3	0.40	C	C	C	C	6	0	0
14	8.62	0.27	13.6	0.42	C	C	C	C	0	++	0
15	46.5	0.21	7.5	0.34	E	E	C	C	25	++	0
16	6.61	0.29	10.6	0.46	E	E	C	C	35	++	+
17	4.71	0.26	11.1	0.62	E	E	C	C	4	+	0
18	9.73	0.54	15.9	0.89	E	E	C	C	0	0	0
19	6.46	0.28	15.5	0.67	E	E	C	C	20	++	0
20	7.61	0.51	15.5	1.03	C	C	C	C	6	+++	0
21	9.73	0.36	18.5	0.68	E	E	C	C	0	0	0
Mean	6.63	0.31	12.6	0.60							
Range	4.57— 9.73	0.21— 0.54	7.5— 18.5	0.34— 1.03							
22	7.43	0.30	8.4	0.34	0	C	0	C	0	0	0
23	6.78	0.23	15.7	0.54	E	E	0	C	0	0	0
24	6.82	0.28	9.1	0.38	C	C	0	C	2	0	0
Mean	7.01	0.27	11.1	0.42							

The weight of the heart and the lungs of most of the rabbits exceeds that of the control rabbits of corresponding sizes. The percentage of these of the weight at death is shown in the table, the deviation from the control material being especially clear. Since most rabbits with heart failure showed a considerable decrease in weight, and since it is not likely that this is associated with the weight of the heart or the lungs, the weights of these two organs have been calculated as percentages of the operation weight. The averages are: heart 0.27 (range 0.19—0.39) and lungs 0.52 (range 0.31—0.90). This, too, indicates the mean increase in the weight of the heart and lungs. It does not correlate clearly with the time the animals survived after operation.

The observations made in vivo of those rabbits who developed hypertonia but did not show symptoms of heart failure (IR 22—24) while alive, are presented in Table 8.

In rabbit IR 22 a post-operational decrease in weight is discernible but not to the same extent as in most of the rabbits in the preceding group. Picture 3 shows the findings made in vivo of rabbit IR 23.

Table 10 presents the post-mortem observations. These revealed unexpected congestion in the liver and lungs.

STROPHANTHIN-TREATED RABBITS WITH HEART FAILURE

The observations in vivo are presented in Table 11. As in the preceding group (untreated rabbits with heart failure), a decrease in weight and an increase in blood pressure could be detected. Rabbit SIR 1 died spontaneously during the first twenty-four hours after operation. After the animals developed symptoms of heart failure, their treatment with strophanthin proceeded in the following way:

SIR 1. Laboured respiration and orthopnea were diagnosed 8 hrs. after the compression of the aorta. Received strophanthin, 0.03 mg/kg in one dose, which manifestly eased respiration. Died spontaneously 8 hrs. later.

SIR 2. Began to develop symptoms of heart failure 4 days after operation. Received 0.02 mg/kg of strophanthin 5 times (at 12, 22, 2 and 6 hr. intervals). After each dose, respiration became easier and orthopnea disappeared. Was killed 2½ hrs. after the last dose of strophanthin.

SIR 3. Developed symptoms of heart failure 6 days after operation. Received 0.02 mg/kg of strophanthin twice, at a 5½ hr. interval. Respiration became easier both times. Was killed 2¼ hrs. after the last dose of strophanthin.

SIR 4. Symptoms of heart failure 7 days after operation. Received 0.02 mg/kg of strophanthin 3 times (at 6 and 16 hr. intervals). The condition of the animal improved as above. Was killed 7 hrs. after the last dose of strophanthin.

SIR 5. Symptoms of heart failure 5 days after operation. Worsened in 4 days and the strophanthin treatment was begun. Received 0.02 mg/kg 3 times (at 8 and 16 hr. intervals). Condition improved each time, as in the preceding cases. Was killed 8 hrs. after the last dose of strophanthin.

TABLE 11

IN VIVO FINDINGS OF RABBITS WITH HEART FAILURE TREATED WITH STROPHANTHIN

Sex, weight at beginning of observation, observation period before compression of the aorta, weight at operation, change in weight, survival time after operation, weight at death, change from operation weight, blood pressure before operation, maximum blood pressure measured, blood pressure before death.

Rabbit 1 died spontaneously, the rest were killed.

SIR	Sex	Weight at beginning of observation g	Observ. time days	Weight at operation g	Change in weight per cent	Survival time days	Weight at death g	Change in weight per cent	Blood pressure		
									before oper.	max. mmHg	before death
1	♂	2 690	4	2 700	+0.4	1	2 700	0	100—110		
2	♂	3 290	5	3 230	—1.8	7	2 980	— 7.7	120—130	135	135
3	♂	3 190	4	3 140	—1.6	7	2 730	—13.1	80— 95	170	170
4	♂	2 340	3	2 340	0	8	2 180	— 6.8	110	155	155
5	♂	2 830	4	2 640	—6.7	10	2 410	— 8.7	125—130	230	225
6	♀	1 570	3	1 470	—6.4	11	1 430	— 2.7	90	145	140
Mean		2 652	4	2 587	—2.7	7	2 405	— 6.5	107	167	165
Range		1 570— 3 290	3—5	1 470— 3 230	—6.7— +0.4	1—11	1 430— 2 980	± 0 — —13.1	80—130	135— 230	135— 225

TABLE 12

POST-MORTEM FINDINGS OF RABBITS WITH HEART FAILURE TREATED WITH STROPHANTHIN

Heart and lung weights and their percentage of whole weight, macroscopically and microscopically observed congestion and the appearance of fluid in the pleural and peritoneal cavities and subcutaneously.

SIR	Heart		Lungs		Macr. obs.	Micr. obs.	Liver		Fluid		
	g	per cent	g	per cent			macr. obs.	micr. obs.	pleur.	perit. ml	subcut.
1	5.65	0.21			C	C	C	C	8	++	0
2	5.38	0.18	10.2	0.34	C	C	C	C	0	++	0
3	9.76	0.36	29.4	1.08	E	E	C	C	40	+++	0
4	4.80	0.22	5.8	0.27	C	C	C	C	0	0	0
5	6.10	0.25	7.6	0.32	E	E	C	C	8	+	0
6	4.35	0.31	12.6	0.88	E	E	C	C	2	++	0
Mean	6.01	0.25	13.1	0.58							
Range	4.35— 9.76	0.18— 0.36	5.8— 29.4	0.27— 1.08							

SIR 6. Symptoms of heart failure 8 days after operation. Worsened within 2 days. Received 0.02 mg/kg of strophanthin 3 times (at 7½ and 16 hr. intervals). Respiration grew easier each time and the animal more vigorous. Was killed 9 hrs. after the last dose of strophanthin.

Table 10 presents the heart rates of the rabbits. In four cases (SIR 3—6) strophanthin caused a considerable decrease in the rate, in one (SIR 2) the decrease was slight. None of the rabbits showed an increased rate, as did one of the control rabbits (SCR 1, Table 5) which was treated with strophanthin doses of corresponding size, and three out of the four control rabbits that were treated with larger doses of strophanthin.

Table 12 presents the results of the post-mortem examinations made of the same rabbits. All of them were found to have congestion in the lungs and liver. Most of them had fluid in the pleural and peritoneal cavities, too.

THE ACh CONTENT OF THE HEART

UNTREATED CONTROL RABBITS

The aim was to determine the ACh content in both auricles and ventricles and in the interventricular septum. The results are shown in Table 13.

TABLE 13

THE HEART ACh CONTENT OF CONTROL RABBITS (μg/g)

CR	RA	LA	RV	LV	IVS
5	1.65	0.66		<0.05	0.18
6	1.72	0.88	<0.06	<0.06	<0.08
7	1.00	1.20	0.31	0.07	0.40
8	1.46	1.49	<0.06	<0.03	0.14
9	0.97	0.40	<0.22	<0.05	0.09
10	2.52	1.12	0.40		0.83
Mean	1.55 ±0.57[1]	0.96 ±0.39			0.27

The content of the right auricle is either larger than that of the left or as large (within the limits of error of the method). Unfortunately the content of the ventricles was usually impossible

[1] The ± value in this and the following tables refers to standard deviation.

to determine; on the other hand the sensitivity of the method was sufficient to investigate the interventricular septum in 5 cases out of 6.

STROPHANTHIN-TREATED CONTROL RABBITS

Only the contents of the auricles were determined. The results of the rabbits treated with the treatment doses of strophanthin for rabbits with heart failure are shown in Table 14.

TABLE 14

THE ACh CONTENT OF THE AURICLES OF CONTROL RABBITS TREATED WITH STROPHANTHIN (μg/g)

SCR	RA	LA
1	2.00	0.79
2	1.03	1.00
3	0.65	0.44
4	2.09	1.32
Mean	1.44 ± 0.71	0.89 ± 0.37
5	0.62	0.30
6	0.42	0.39
7	0.59	0.60
8	0.65	0.46
Mean	0.57 ± 0.10	0.44 ± 0.13

The ACh content of the right auricle of SCR 3 was lower than in the rest of the rabbits in the group and in the untreated control rabbits. The rest of the values fall within the range of untreated controls mentioned earlier. The means RA 1.44 ± 0.71 and LA 0.89±0.37 μg/g do not vary significantly from the mean of the control series. On the other hand, the ACh content of the auricles of the control rabbits treated with larger strophanthin doses are statistically significantly smaller than in the untreated series, and the contents of the right auricle smaller than in the control rabbits treated with smaller strophanthin doses. The results are shown in Table 14.

UNTREATED RABBITS WITH HEART FAILURE

Usually only the auricle content was determinable, the content of the interventricular septum in only a few cases. Because of this,

the content of the latter is not compared statistically with the control series. The results are shown in Table 15.

The range of the auricle ACh content is extensive, the means being RA 0.67 ± 0.65 and LA 0.69 ± 0.90 μg/g. Rabbit IR 3 has also been included in this group, although it died within 24 hours of operation. The operated rabbits which did not show signs of heart failure in vivo have not been included. When comparing the

TABLE 15

THE HEART ACh CONTENT OF UNTREATED RABBITS WITH HEART FAILURE (μg/g)

IR	RA	LA	RV	LV	IVS
3	0.48	<0.22			
7	<0.002	0.03	0	0	0
8	0.96	1.39	0.31	<0.10	0.17
9	2.43	3.21	0.55	0.13	0.18
10	0.31	1.17	<0.09	<0.03	<0.06
11	1.12	0.38			<0.10
15	0.87	0.61			
16	0.49	<0.22	0.13	<0.05	<0.06
18	<0.58	<0.41			
19	0.52	0.40			<0.16
20	0.06	0.06		<0.01	<0.01
21	0.20	<0.13	<0.09	<0.09	<0.09
Mean	0.67 ± 0.65	0.69 ± 0.90			
22	1.39	0.61			
23	5.27	1.35			
24	1.27	0.58			
Mean	2.64	0.85			

control series with the Wilcoxon test, it is observed that the right auricle content is lower in these rabbits, and the left auricle content in rabbits which survived for at least 6 days after operation.

In the untreated control rabbits the ACh content of the right auricle does not correlate with the weight of the heart, as can be seen from Fig. 5. On the other hand, in the rabbits with heart failure it is confirmed that the ACh content of the right auricle in six of the largest hearts is smaller than normal; of the other five shown in the figure, only one value is clearly smaller than the lowest ACh content of the right auricle of the control rabbits.

Fig. 6 shows the ACh content of the right auricle as a function

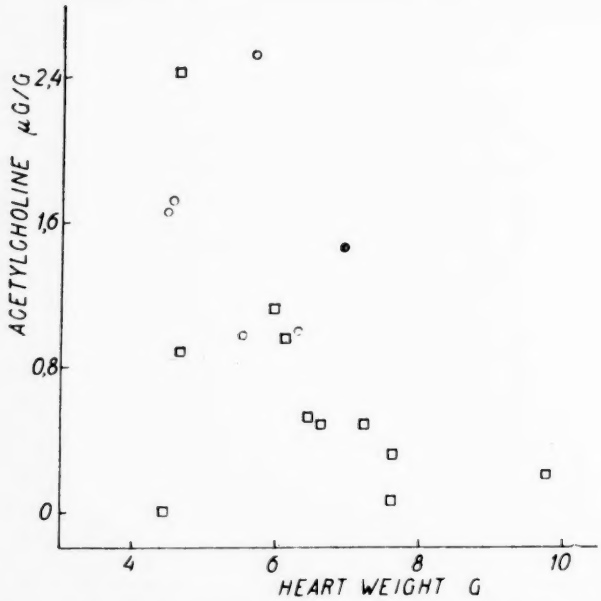

Fig. 5. — The ACh content of the right auricle of normal rabbits and of untreated rabbits with heart failure as a function of the heart weight.

Abscissa: The heart weight in grams.

Ordinate: The ACh content of the right auricle in micrograms per gram of auricular tissue.

○ = control rabbit, □ = rabbit with heart failure.

of the post-operative survival time. It can be seen that in the rabbits that survived for more than a week after operation the value is clearly below the normal range. In one of the rabbits (IR 18), which survived for 12 days, the ACh content of the right auricle could not be determined because of the poor sensitivity of the leech preparation used. Consequently it has been omitted from the picture. In any case it was below 0.58 µg/g and thus clearly below the normal range.

Fig. 7 shows the ACh content of the right auricle as a function of the heart rate just before killing. A clear correlation can be seen: the less the heart rate, the greater the ACh content in the right auricle. The ACh content of the left auricle does not show a similar correlation, no relation could be detected between it and the heart rate. In this respect the rabbits with heart failure treated with strophanthin behaved like the untreated ones, except SIR 2, in which the ACh content is greater than in the others as regards the

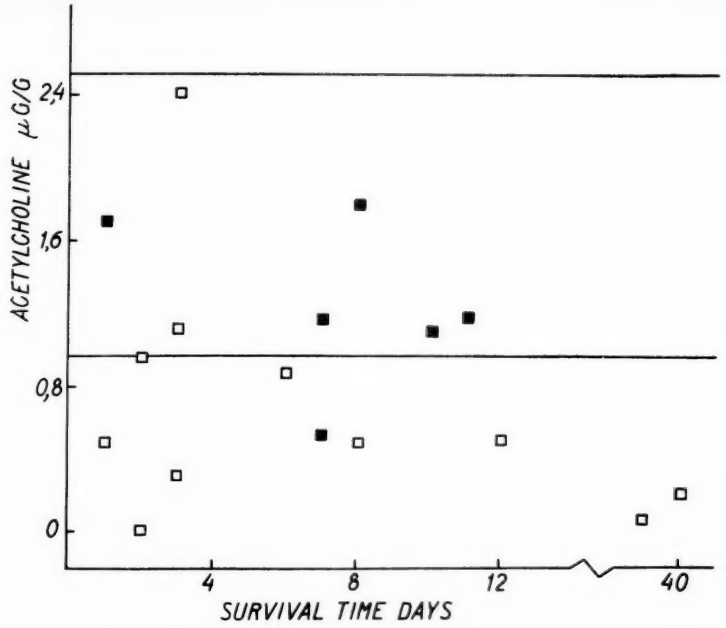

Fig. 6. — The ACh content of the right auricle of untreated rabbits and rabbits with heart failure treated with g-strophanthin as a function of the survival time after compression of the aorta.

Abscissa: Survival time in days after compression of the aorta.
Ordinate: ACh content of the right auricle in micrograms per gram of auricular tissue. The lines in the direction of the abscissa indicate the lower and upper limits of the range in the ACh content of normal rabbits.

□ = untreated rabbits with heart failure.
■ = rabbits with heart failure treated with strophanthin.

heart rate. This could be explained on the basis of the dual effect of strophanthin in changing heart rhythm, a fact which appears clearly in the rabbits treated with strophanthin. Here no correlation between the rate of the heart and the ACh content of the right auricle could be determined with either of the doses used.

The blood pressure did not correlate with the ACh content of either auricle.

In none of the three rabbits with hypertonia in which no heart failure was observed in vivo, but in which congestion in the lungs and liver was noticed at post-mortem the ACh content of the auricles fell below the normal range. On the contrary, the ACh content of the auricle of IR 23 was greater than that of any of the rabbits examined. The results are shown in Table 15.

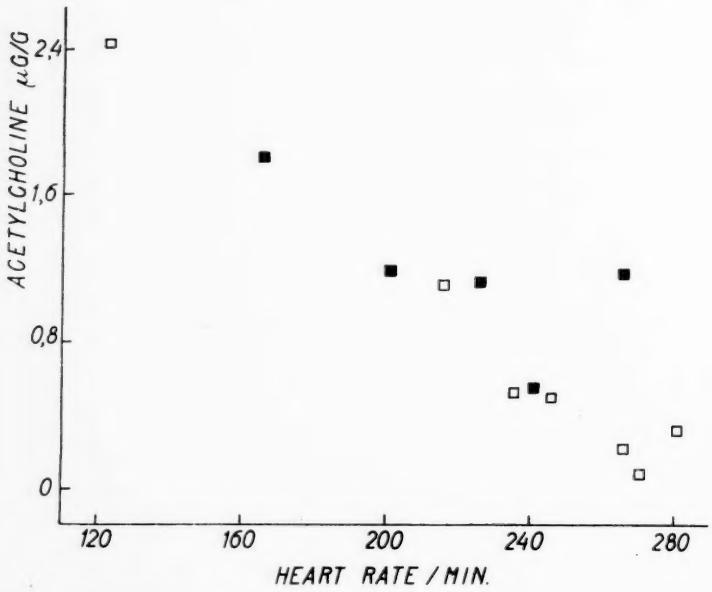

Fig. 7. — The ACh content of the right auricle of untreated rabbits with heart failure and of those treated with strophanthin as a function of the heart rate. For explanation of symbols, see Fig. 6.

The heart rates of these two rabbits (IR 23 and 24) were registered before the animals were killed. They were 230 and 245/min. and did not correlate with the ACh content of the right auricle, as was the case with the previous group of rabbits.

STROPHANTHIN-TREATED RABBITS WITH HEART FAILURE

The results are presented in Table 16. The ACh content of both auricles in five of the six rabbits in this group fall within the normal range, the mean values do not differ significantly from the corresponding mean values of the control rabbits. In rabbit 3, the ACh content is lower than normal in both auricles, as is the case with the majority of the untreated rabbits with heart failure. Here, too, there were symptoms of heart failure in vivo and the congestive changes detected post-mortem were very clear.

It appears from Table 9 that strophanthin retarded the heart rate in all the rabbits with heart failure examined, this effect being

TABLE 16

THE ACh CONTENT OF THE AURICLES OF RABBITS WITH HEART FAILURE TREATED WITH STROPHANTHIN (μg/g)

SIR	RA	LA
1	1.71	0.67
2	1.17	0.81
3	<0.53	<0.13
4	1.80	0.69
5	1.11	0.46
6	1.18	1.49
Mean	1.25 ± 0.46	0.71 ± 0.45

variable in normal rabbits. The auricular ACh content of the control rabbits treated with small amounts of strophanthin did not vary significantly from the contents of the control rabbits. On the other hand, the rabbits with heart failure treated with similar doses of strophanthin have statistically significantly greater ACh content in the right auricle than the untreated rabbits with heart failure.

STATISTICAL TREATMENT

Table 17 summarizes a comparison made by the Wilcoxon test of the auricular ACh content in the different rabbit groups.

TABLE 17

STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN THE ACh CONTENT IN THE HEARTS OF THE RABBIT GROUPS EXAMINED

If the random probability P has exceeded the value 0.05, then the difference is statistically not significant and two dots (..) are substituted for P.

The groups compared	RA	LA
Untreated rabbits with heart failure (IR)/Control rabbits (CR)	0.01	..
IR'S that survived for at least 6 days/CR's	0.01	0.05
Rabbits with heart failure treated with strophanthin (SIR)/CR's	..	..
SIR'S/IR'S	0.05	..
Control rabbits treated with small stroph. doses (SCR)/ Control rabbits (CR)	..	..
Control rabbits treated with large stroph. doses (SCR)/ Control rabbits (CR)	0.01	0.05
Control rabbits treated with large stroph. doses/Control rabbits treated with small stroph. doses	0.05	..

THE ChE ACTIVITIES OF THE HEART

UNTREATED CONTROL RABBITS

MCh, BuCh and ACh have been used as substrates. The enzyme activity has been reported as μl CO_2 per hour per gram.

MCh as substrate. — The results obtained are given in Table 18.

TABLE 18

THE HEART ChE ACTIVITY OF CONTROL RABBITS, MCh AS SUBSTRATE (μl CO_2/hr./g)

If parallel determinations have been made, both figures have been given and also the mean value.

CR	RA	LA	RV	LV	IVS
1	423		253 259	453	253
			265		
2		228 297	240 267	168	250 267
		365	293		283
3	403 469	438		113 119	293 304
	535			125	310
4	438 482		0 0	445	255
	525		0		
5	623	315	308	70 85	348
				100	
7	530 545	675	93	0 0	
	560			0	
Mean	508	431	185	212	285

The order of magnitude of the activity of the different parts of the heart was as follows: RA — LA — IVS — LV — RV.

BuCh as substrate. — Because ChE of the heart has been found to be mainly non-specific and more attention has been paid to it in earlier investigations (*e.g.* Govier *et al.* 1953), the majority of the ChE determinations in this work, too, have been done by using a substrate of the non-specific ChE as substrate.

The results are given in Table 19.

The activities obtained from different heart specimens vary greatly, so that a clear difference is manifest only between the auricles and ventricles.

ACh as substrate. — Unfortunately the amount of auricular tissue was only sufficient to make a few determinations with this "natural" substrate. However, some values are presented,

TABLE 19

THE HEART ChE ACTIVITY OF CONTROL RABBITS, BuCh AS SUBSTRATE
(μl CO_2/hr./g)

CR	RA	LA	RV	LV	IVS
2	1 638	1 545	730 817	378	610 630
			903		650
3		1 240		345 388	463 477
				430	490
4			1 313	655 742	800 938
				828	1 075
5		1 443	635	363	370 417
					463
6	1 918 1 981	2 475 2 605	1 600		
	2 043	2 735			
8		1 740	865	548 584	418 442
				620	465
10	873		678	753 763	530
				773	
11	1 298	655 697	853	480 483	673 716
		758		485	758
12	2 098 2 138	1 330 1 339	518	638 659	468 541
	2 178	1 348		680	613
Mean	1 568	1 516	910	545	586

TABLE 20

THE HEART ChE ACTIVITY OF CONTROL RABBITS, ACh AS SUBSTRATE
(μl CO_2/hr./g)

CR	RA	RV	LV	IVS
2		848 852	520 530	720
		855	540	
3			280	545 584
				623
4	1 600	698 699	395	780 813
		700		845
6	1 328	750 775	750 773	
		800	795	
7		530 538	308 313	200 223
		545	318	245
9	1 698 1 736	1 280 1 334	925 975	
	1 773	1 388	1 025	
Mean	1 555	839	544	585

mainly as a comparison with the results obtained with BuCh as substrate.

The results are given in Table 20.

It can be observed throughout that the hydrolysis of ACh is of the same magnitude as that of BuCh.

STROPHANTHIN-TREATED CONTROL RABBITS

BuCh has been used as a substrate.

The results of the control rabbits treated with the doses administered to the rabbits with heart failure are shown in Table 21.

TABLE 21

THE ChE ACTIVITY OF THE AURICLES AND THE INTERVENTRICULAR SEPTUM OF CONTROL RABBITS TREATED WITH STROPHANTHIN, BuCh AS SUBSTRATE

(μl CO_2/hr./g)

SCR	RA		LA		IVS	
1	820	847	1 883	2 096		
	873		2 308			
2		988	2 230	2 380	495	507
			2 530		518	
3		1 418	1 318	1 453	583	628
			1 588		673	
4	1 165	1 175	1 383	1 451	615	652
	1 185		1 518		688	
Mean		1 107		1 845		596
5	2 068	2 183	2 263	2 318	1 663	1 777
	2 298		2 373			
6		958	1 295	1 327	565	577
			1 358		588	
7		740	1 610	1 674	665	745
			1 738		825	
8		1 030		1 770		633
Mean		1 228		1 771		983

No statistically discernible difference in regard to the control series appeared in any of the parts of the heart studied (RA, LA and IVS). However, each value for the right auricle is below the average for the control series.

When the rabbits were treated with larger doses of strophanthin,

the non-specific ChE activity of the heart did not differ from normal either. In three out of four rabbits (SCR 6, 7 and 8), the activity in the right auricle was smaller than the mean of the control group. On the other hand, in rabbit 5, in which toxic symptoms were clearer, great activity was noted. The results are presented in Table 21.

The activity in the left auricle fully corresponds to the control series. On the basis of this, the decrease in the ACh content of the auricles caused by toxic strophanthin doses cannot be regarded as deriving from the increased activity of the non-specific ChE.

UNTREATED RABBITS WITH HEART FAILURE

ChE determinations have been made of the rabbit group that survived for 2—40 days after compression of the aorta and in which, in vivo, there were symptoms of heart failure, using MCh, BuCh and ACh as substrates. For the rabbits which died within 24 hours of compression of the aorta and in which heart failure was observed in vivo, BuCh only was used.

MCh as substrate. — The results of the rabbits that survived for 2—40 days after compression of the aorta are shown in table 22.

TABLE 22

THE HEART ChE ACTIVITY OF RABBITS WITH HEART FAILURE THAT SURVIVED 2—40 DAYS AFTER COMPRESSION OF THE AORTA, MCh AS SUBSTRATE

(μl CO_2/hr./g)

IR	RA	LA	RV	LV	IVS
8	465	308 329	38 59	175	185 217
		350	80		248
9	293		182 202	0	
			215		
12	475 480		310 317	0 0	135 143
	485		323	0	150
13	513	625	405	275	268 281
					293
14	150	295 305	260 269	195 204	85 114
		315	278	213	143
17	505 568	225	280 348	115 135	103 116
	630		415	155	130
20	443 467	540 577	175	400 437	155
	490	613		473	
Mean	419	412	255	175	171

In rabbit IR 14 a noticeably small value of the right auricle can be observed. Otherwise the values of the auricles and ventricles do not differ essentially from the values of the control series. On the other hand, the activity of the interventricular septum is statistically significantly smaller than in the control material. Obviously, therefore, the activity of the specific ChE cannot explain the decrease in the ACh content in the auricles of rabbits with heart failure.

BuCh as substrate. — The results of the rabbits that died during the first day after operation are given in Table 23.

TABLE 23

THE HEART ChE ACTIVITY OF RABBITS WITH HEART FAILURE THAT SURVIVED LESS THAN TWO DAYS AFTER COMPRESSION OF THE AORTA, BuCh AS SUBSTRATE

(μl CO_2/hr./g)

IR	RA	LA	RV	LV	IVS
1	2 365 2 504	750	838 868	1 278 1 291	
	2 643		900	1 303	
2	433 446	1 440 1 600	740	135	715 732
	458	1 760			748
3	1 115	928	463	418	540
4	1 030 1 133		493	373	238 287
	1 235				335
5	1 535 1 612				
	1 688				
6	1 675 1 717	1 608	690	705	903
	1 758				
Mean	1 421	1 222	651	584	615

In none of the parts of the heart studied was there significant difference in regard to the control series.

The results of the rabbits that survived for 2—40 days after compression of the aorta are presented in Table 24.

A statistically significant difference from the control series occurs only in the interventricular septum in which the non-specific ChE activity is greater than in the control material. According to this, the non-specific ChE activity has not increased in a way that would explain the decrease in the ACh content of the failing heart (c.f. also fig. 8).

The results of the rabbits in which there was hypertonia but no discernible symptoms of heart failure in vivo are presented in Table 24.

TABLE 24

THE HEART ChE ACTIVITY OF RABBITS WITH HEART FAILURE THAT SURVIVED 2—40 DAYS AFTER COMPRESSION OF THE AORTA, BuCh AS SUBSTRATE (μl CO_2/hr./g)

IR	RA		LA		RV		LV		IVS	
7		2 668			1 498	1 538	1 255	1 293	1 743	1 868
					1 578		1 330		1 992	
8			2 985	3 257		620	678	766		1 558
			3 528				853			
9		1 663		2 823		1 075	608	628	575	645
							648		715	
10				2 383	335	388	360	400		
					440		440			
11	1 923	1 997	1 550	1 618		780	433	477		1 083
	2 070		1 685				520			
12	900	952	628	654	835	868		253		
	1 003		680		900					
13		1 758		853	575	583		848	785	860
					590				935	
14	1 375	1 457		2 407		703	1 193	1 207	615	700
	1 538						1 220		785	
16		1 338		1 045		1 030	1 015	1 043		760
							1 070			
17		1 100		1 143	818	827	1 033	1 038	730	842
					835		1 043		953	
18		3 130	2 950	2 950	755	829	480	527		1 800
			2 950		903		575			
19	1 888	1 889		2 085	635	642		403	918	987
	1 890				648				1 055	
20		1 945	2 540	2 547		360	445	497		638
			2 555				548			
21	1 575	1 633	1 830	1 872	478	521	555	597		1 355
	1 690		1 913		563		638			
Mean		1 794		1 972		768		713		1 091
22		2 055	2 845	2 812	350	408	830	869	325	328
			2 778		465		908		330	
23		1 680		2 305		385		883	1 690	1 800
									1 910	
24		1 583		1 595	465	493	428	452	398	406
					520		475		413	
Mean		1 773		2 237		428		735		845

Throughout, the activity of the right ventricle is lower than in the control group and the activity of the interventricular septum in IR 23 is considerably greater, just as in two of the rabbits in the

former group. It is worth noting that the ChE activity of the right auricle of IR 23, in which the ACh content of the right auricle is greater than the others, does not differ from the control series.

ACh as substrate. — The results of the group that survived for 2—40 days after compression of the aorta are presented in Table 25.

TABLE 25

THE HEART ChE OF RABBITS WITH HEART FAILURE THAT SURVIVED 2—40 DAYS AFTER COMPRESSION OF THE AORTA, ACh AS SUBSTRATE (μl CO_2/hr./g)

IR	RA	LA	RV	LV	IVS
8			365	388 397 405	405
13			505 528 550	393 404 415	405 457 508
14	830 834 838	2 055	323	458 527 595	163 208 253
17		1 228	465 498 530	495 552 608	528 558 588
18	2 718 2 767 2 815	3 233 3 302 3 370			
20		1 555	520 573 625	528 553 578	200 239 278
Mean	1 801	2 035	457	487	373

There are only two determinations of the right auricle, but these already show a great range of values. These values correspond to the hydrolysis of BuCh. On the other hand, the activity of the interventricular septum is of the same class or smaller than in the control rabbits. Thus this corresponds more to the situation when MCh is used as a substrate than when BuCh is used.

STROPHANTHIN-TREATED RABBITS WITH HEART FAILURE

BuCh has been used as a substrate. The results of the determinations made of the auricles and interventricular septum are given in Table 26.

They do not show any statistically significant difference from the values of the control rabbits. On the other hand, the activities of the right auricle and interventricular septum are significantly lower than in the untreated rabbits with heart failure.

When comparing on the one hand the ACh content of the right auricle and its non-specific ChE activity in untreated rabbits with

TABLE 26

THE HEART ChE ACTIVITY OF RABBITS WITH HEART FAILURE TREATED WITH STROPHANTHIN, BuCh AS SUBSTRATE (μl CO_2/hr./g)

SIR	RA		LA		IVS	
1	635	677		1 100	505	545
	718				585	
2	1 063	1 113		1 113	693	699
	1 163				715	
3	1 193	1 371	1 543	1 592	1 258	1 294
	1 548		1 640		1 330	
4		770	1 178	1 207		675
			1 235			
5	1 045	1 234		2 123	513	523
	1 423				533	
6	595	738	1 723	1 756	545	550
	880		1 788		555	
Mean		984		1 482		714

heart failure and on the other hand those treated with strophanthin, it can be seen from Fig. 8 that a smaller ACh content is most generally connected with a greater ChE activity in strophanthin-treated rabbits. This may mean that strophanthin at least partly increases the ACh content of the auricles of failing hearts by checking the non-specific ChE though the latter has not increased significantly in rabbits with heart failure.

STATISTICAL TREATMENT

Table 27 summarizes a comparison made by the Wilcoxon test of the heart ChE activities in different groups of rabbits.

TABLE 27

STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN THE HEART ChE ACTIVITIES IN THE RABBIT GROUPS EXAMINED

Substrate	Groups compared	RA	LA	RV	LV	IVS
MCh	IR's 2—40 days/CR's	..	..	..	..	0.05
BuCh	IR's 2—40 days/CR's	..	..	..	..	0.01
	IR's under 2 days/CR's ..	..	..	..	..	..
	SIR's/CR's	..	..			..
	SIR's/IR's 2—40 days	0.01	..			0.05
	SCR's (small doses)/CR's	..	..			..
	SCR's (large doses)/CR's ..	..	..			..
	SCR's (small doses)/ SCR's (large doses)	..	..			..

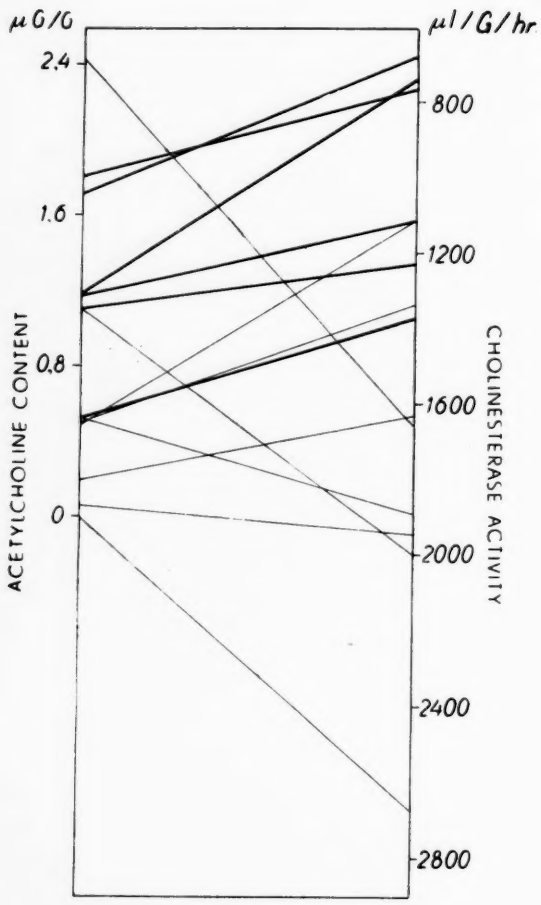

Fig. 8. — The correlations of the right auricular ACh content and the non-specific ChE activity (BuCh as substrate) of strophanthin-treated (thick lines) and untreated (thin lines) rabbits with heart failure.

V. DISCUSSION

It has been proved by Burn and co-workers that the ACh metabolism of the heart has a fundamentally important function on maintaining its rhythmic activity. Depending on circumstances, the ACh effect on the function of the heart is either stimulating or inhibiting. Gremels (1937) and Raab (1956) have supposed that in several types of heart failure some deficiency in the cholinergic system of the heart could be detected. The latter believes that athletes who usually have slow pulse rates, have an unusually active cardiac cholinergic system.

In the present work the ACh content of the auricles, the mean values RA 1.55 $\pm$ 0.57 and LA 0.96 $\pm$ 0.39 μg/g, does not differ essentially from that presented by Vlk, whose figures were RA 1.37 $\pm$ 0.22 and 0.78 $\pm$ 0.11 μg/g. In this work, however, the range of the ACh content is much larger, which may be due to the heterogeneous quality of the rabbit material used.

The ACh content of both auricles of the rabbit heart was found to be lower in cases of heart failure, the decrease being more marked in the right auricle. After g-strophanthin treatment, the ACh content of both auricles of rabbits with heart failure was discovered to be of the same degree of magnitude as that of the control rabbits, while the difference in regard to the untreated rabbits with heart failure was to be seen more clearly in the right auricle. The decrease in the ACh content referred to above was regular in rabbits that survived for more than a week after compression of the aorta. The hearts of all of these were found to have increased in weight. One rabbit (IR 7), which died 2 days after operation and the weight of the heart of which was in the same class asthat of normal hearts, also had a noticeably low auricular ACh content. The ACh contents of the auricles of the four rabbits that died during the first week

after the operation were of the same magnitude or only a little lower than those of normal rabbits. The three rabbits in which no heart failure could be detected in vivo, showed no decrease in the ACh content of the auricles. On the contrary, one of them (IR 23) had an abnormally high ACh content in the right auricle. These three rabbits lived 19, 20 and 83 days respectively after compression of the aorta. Two of them (IR 22 and 24) had hypertrophied hearts, and in all congestion in the lungs and liver was found at the post-mortem examination. The blood pressure of these rabbits was considerably high (maximum 240, 245 and 230 mmHg respectively), more than that of any rabbit that showed symptoms of heart failure while alive.

Alexander and Drury (1957) discovered considerable individual variations between different rabbits in adaptation to an increase in blood pressure. Some survived for months despite hypertonia caused by compression of the aorta. Obviously the three rabbits used in the present series were of this type. It is not impossible that the normality of the ACh content of their auricles was a phenomenon caused by such adaptation, corresponding to Raab's theory of athletes' heart.

The rate of the rat's heart has previously been shown to correlate inversely with its ACh content (Beznák 1956). In the present work the heart rate of the rabbits with heart failure either treated or untreated with g-strophanthin were seen to correlate in the same way with the ACh content of the right auricle. The results obtained indicate that the cholinergic nervous system regulates the heart rate in heart failure. The negative chronotropic influence of a vagus stimulus has been shown to result from its inhibiting influence on the slow diastolic depolarization in the sino-auricular node. This slow depolarization is considered to result from ACh synthesis in the sino-auricular node, and the effect of a vagus stimulus from inhibiting this ACh synthesis in the node (Burn 1956).

In the present work the influence of strophanthin on the heart rate of control rabbits has been found to be variable. Rothlin and Suter (1947) and Bircher, Rothlin and Suter (1947) noticed that k-strophanthin affected the heart rate of the cat in different ways. This did not give any criteria for distinguishing therapeutic and toxic strophanthin doses in normal cats. Cardiac glycosides have an effect on the heart rate because of a vagal stimulation and of a

direct influence on the heart muscle cells. The fact that no inverse correlation could be detected between the heart rate of the strophanthin-treated control rabbits and the ACh content of the right auricles is therefore not surprising.

A choline-free diet has been found to cause bradycardia in rats; a vagus stimulus did not cause any further decrease in their heart rates (Abdon and Borglin 1946). If the "endogenous" ACh had decreased, bradycardia and a direct correlation between the ACh content and the heart rate could have been expected in this work. A similar phenomenon appeared in the experiments of Burn and co-workers described earlier: the decrease in the ACh synthesis was accompanied by a decrease in the rate of the auricles.

The auricles have proved more interesting than the ventricles when studying the effects of ACh on the heart. If, however, ACh is an essential factor in the mechanism that releases the contraction of a muscle cell, a further study of the ACh metabolism of the ventricles would be as important as that of the auricles. Heart failure is actually a failure of a ventricle or of the ventricles. In the present work the ACh content of the ventricles of most control rabbits was unfortunately too low to be determined even by the most sensitive specific method of ACh assay available.

The fall in the ACh content could be supposed to result from a decrease in its synthesis or an increase in its hydrolysis, or from both these factors operating simultaneously. Correspondingly, the effect of cardiac glycosides that sensitize the heart to a vagus stimulus and ACh can result from the increase in ACh synthesis or the prevention of hydrolysis caused by them. No statistically significant increase in the ChE activity of the auricles or ventricles of rabbits with heart failure could be detected, as far as the specific ChE or non-specific ChE were concerned, except in the interventricular septum, where the activity of the non-specific ChE was higher than that of the control rabbits. On the other hand, the specific ChE activity was lower than that of the control material. This is possibly due to a conduction disturbance in the specialized conductive tissue, a matter which has not yet been analyzed.

In this work there is no significant increase in the specific or non-specific ChE activity that could explain the decreased contents of ACh in the auricles of rabbits with heart failure. Apart from rabbit IR 2, in which the figures for the right auricle and left

ventricle were lower than in the others, the figures of the left ventricle in particular correspond well with the control material. Therefore the suggestion advanced by Govier and co-workers (1953) as to an inhibition of the non-specific ChE of the left ventricle of a failing heart cannot be confirmed.

The effect of g-strophanthin on the non-specific ChE of the right auricle is statistically significant. The same effect is also found in the interventricular septum. Perhaps this partly explains the normality of the ACh content of the auricles of the strophanthin-treated rabbits with heart failure.

When normal rabbits were given similar doses of g-strophanthin as those given to rabbits with heart failure, the ACh content of the right auricle was lower than the control values in one case out of four. In the other cases it remained within the normal limits. On the other hand, when larger, clearly toxic strophanthin doses were used, the ACh content of the right auricles of all four rabbits examined was found to be lower than the control values.

g-Strophanthin seems to tend to decrease the activity of the non-specific ChE of the right auricle; it was lower than the average of the control series in seven cases out of eight. Only in one case (SCR 5) was the ChE activity higher than normal throughout. This tendency to inhibit ChE cannot be found in the left auricle. This, again, indicates that g-strophanthin in toxic doses inhibits both ACh synthesis and hydrolysis, while, in therapeutic doses it inhibits hydrolysis and possibly improves synthesis (c.f. Govier *et al.* 1953).

ACh has been found to decrease the toxic effect of strophanthin on the heart (Chatterjee 1954; Holland, Greig and Dunn 1954). Holland, Dunn and Greig (1952) explained the effect of glycosides on the ion-interchange and ECG as resulting from their influence on the ACh metabolism of the heart. It is a fact that hypopotassemia increases the toxicity of cardiac glycosides and that potassium decreases their toxicity (*e.g.* Lown *et al.* 1951). Toxic glycoside doses are known to decrease and therapeutic doses to increase the intra-cellular potassium content (Hagen 1939; Dunn, Holland and Greig 1954). This may be due to the different influences of various glycoside doses on the ACh metabolism of the heart.

VI. SUMMARY

In thirty rabbits the aorta was compressed at a point between a. coeliaca and a. mesenterica cranialis. Of these, six showed symptoms of heart failure during the first day after operation and fifteen from 2—40 days after compression of the aorta. In three rabbits no symptoms of heart failure appeared in vivo during an observation period of 19, 20 and 83 days, despite increased blood pressure. Six rabbits in which signs of heart failure were discerned were treated with g-strophanthin. The diagnostic criteria for heart failure in vivo were laboured breathing, orthopnea, cyanosis and decreasing appetite, and at post-mortem examination, macroscopic and microscopic congestive changes in the lungs and liver and fluid gatherings. The blood pressure was measured from the "Carotis loop", the heart rate electrocardiographically. 20 rabbits were used as the control series; of these, eight were treated with g-strophanthin — four with doses corresponding to those given to the rabbits with heart failure, and four with obviously toxic doses.

In the series, the ACh content and the ChE activity were studied in the right and left auricles, the ventricles and the interventricular septum. MCh, BuCh and ACh were used as substrates in the gasometric ChE determinations.

The ACh content in the auricles was found to decrease in the majority of rabbits with heart failure. This was the more regular the longer the post-operative survival time. After the g-strophanthin treatment the ACh content in the auricles was within normal limits in five cases out of six.

In both the untreated and strophanthin-treated rabbits with heart failure there was a correlation between the ACh content of the right auricle and the heart rate. It was not found that g-strophanthin had any clear effect on the heart ACh content of normal

rabbits when given doses equivalent to those given to rabbits with heart failure;toxic doses clearly decreased the ACh content of both auricles. In these the heart rate did not correlate with the ACh content of the right auricle.

The ChE activity of the untreated rabbits with heart failure only showed a statistically significant difference from the control rabbits as regards the interventricular septum; here the specific ChE activity was found to be lower than normal and the non-specific higher. In the rabbits with heart failure treated with g-strophanthin the non-specific ChE of the right auricle was significantly lower than in the untreated ones. A similar tendency also appeared in the control rabbits treated with strophanthin.

The results confirm that the ACh metabolism of the right auricle regulates the heart rate in heart failure and the lowering effect of g-strophanthin on the heart rate depends on the increase in the ACh content it creates in this part of the heart. This could, at least partly, depend on the inhibiting effect of strophanthin on the non-specific ChE. The significance of ACh metabolism in adapting the heart to greater strain and its defectiveness in heart failure is discussed.

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